AN ASSESSMENT OF HEAVY METAL CONTAMINATION IN THE MARINE SEDIMENTS OF LAS PERLAS ARCHIPELAGO, GULF OF PANAMA

By

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Table of Contents

Abstract..... i
Acknowledgements..... ii

Chapter 1. – Introduction

1.1. – Preface..... 1 - 3
1.2. – Aims and objectives..... 4
1.3. – Metals as biomonitors..... 5- 6
1.4. – Panama history..... 7 - 9
1.5. – Site description..... 10 - 12

Chapter 2. – Literature Review

2.1. – Case studies..... 13 - 20
2.2. – Possible inputs..... 21 - 25
2.3. – Influencing factors..... 26 - 29
2.4. – Metals in sediments..... 30 - 33
2.5. – Metals in mangroves..... 34 - 35
2.6. – Metal speciation and toxicity..... 36 – 43

Chapter 3. – Methods and Materials

3.1. – Grain size..... 44 - 46
3.2. – Sampling Methods..... 47 - 50
3.3. – Particle Size Analysis..... 51 - 52
3.4. – Metal Analysis..... 53 - 54

Chapter 4. – Results

4.1. – Particle Size Analysis..... 55
4.2. – Metal Analysis..... 56 - 59

Chapter 5. – Discussion

5.1. – Interpretation of results.....60 - 72
5.1.1. Mean Concentrations..... 60 – 64
5.1.1.1. Cadmium..... 60
5.1.1.2. Nickel..... 61
5.1.1.3. Copper..... 61
5.1.1.4. Chromium..... 61 – 62
5.1.1.5. Zinc..... 62
5.1.1.6. Manganese..... 62
5.1.1.7. Lead..... 63
5.1.1.8. Iron..... 63 – 64

5.1.2.. Transect Concentrations..... 65 – 69
5.1.3. Concentrations and depth..... 70 – 71
5.1.4. Particle Size Analysis..... 72 - 73

5.2. – Conclusions..... 74
5.3. – Future studies..... 74

Chapter 6. - References

6.1. – References Cited..... 75 - 81

Chapter 7. – Appendices

7.1. – Appendix 1..... 82 – 84
7.2. - Appendix 2..... 85 - 109

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ABSTRACT

Concentrations of eight heavy metals were examined from the marine sediments of the Archipelago of Las Perlas, the Bay of Panama and separating waters. This study was conducted to detect any contamination levels within the sediments. Concentration levels were found to be in the order of Cd >Ni>Cu>Cr>Zn>Mn>Pb>Fe with the exception that within Panama Bay Cr is seen to have a larger concentration than Cu. Natural background levels of Cd, Ni, Cu, Cr and Zn were observed whereas the concentrations of Mn, Pb and Fe were observed to be bordering on contamination. No significant relationship was found either between each metal or concentration and depth. It was, however, observed that the fine grained fraction of sediment, namely the silt – clay fraction, facilitates the uptake of metals more so than any other grain size.

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1.1. PREFACE

Heavy meals are one of the more serious pollutants in our natural environment due to their toxicity, persistence and bioaccumulation problems (Tam & Wong, 2000).
Trace metals in natural waters and their corresponding sediments have become a significant topic of concern for scientists and engineers in various fields associated with water quality, as well as a concern of the general public. Direct toxicity to man and aquatic life and indirect toxicity through accumulations of metals in the aquatic food chain are the focus of this concern.

The presence of trace metals in aquatic systems originates from the natural interactions between the water, sediments and atmosphere with which the water is in contact. The concentrations fluctuate as a result of natural hydrodynamic chemical and biological forces. Man, through industrialisation and technology, has developed the capacity to alter these natural interactions to the extent that the very waters and the aquatic life therein have been threatened to a devastating point.
All of these issues will be explored further in the following chapters.

The activity of trace metals in aquatic systems and their impact on aquatic life vary depending upon the metal species. Of major importance in this regard is the ability of metals to associate with other dissolved and suspended components. Most significant among these associations is the interaction between metals and organic compounds in water and sediment. These organic species, which may originate naturally from process such as vegetative decay or result from pollution through organic discharge from municipal and industrial sources, have a remarkable affinity and capacity to bind to metals. This phenomenon would naturally alter the reactivity of metals in the aquatic environment. (Signer, 1974).

Many human activities (e.g.; mining, overuse of chemicals, industrial waste from ports and refineries) have a negative impact on several biological processes and there is no doubt that these will continue to affect the functioning of highly productive coastal ecosystems. Contamination caused by trace metals affects both ocean waters, those of the continental shelf and the coastal zone where, besides having a longer

residence time, metal concentrations are higher due to input and transport by river runoff and the proximity to industrial and urban zones (various authors quoted in Guzman & Garcia, 2002).

Trace metals, including those defined as "heavy", arising from industrial and mining activities are discharged into coastal waters and estuaries at many sites. The term **heavy metal** refers to any metallic chemical element that has a relatively high density and is toxic, highly toxic or poisonous at low concentrations. These anthropogically derived inputs can accumulate in local sediments (up to five orders of magnitude above the overlying water Bryan & Langston, 1992) and invertebrates living on or in food, and the rate of accumulation caries widely between species and heavy metal concentration found in "clean" conditions. Less is known of the uptake of these metals by ingestion with food or from close contact with contaminated sediments (Harris & Santos, 2000).

For some time, there has been serious concern about the simultaneous input of unwanted trace elements, present in these mineral fertilisers, like Cd or Cr. These trace metals are much more likely available to biota than those amounts bound to the soil (Sager, 1997).

Approximately 80% of total chromium from mineral fertilizers emanates from basic slag and basic slag potash. Regional differences in application rates and crops lead to differences in trace element loads per farmed area up to 6-fold. Further on, inputs from fertilizers have been compared with input by atmospheric deposition. As a source of lead and cadmium, long-range transport via the atmosphere supersedes the input from mineral fertilizers, whereas in case of chromium it is reverse.

It is widely recognised that marine ecosystems can become contaminated by trace metals from numerous and diverse sources. However, anthropogenic activities, such as mining and industrial processing of ores and metals, still remain the principal cause of the increased amount of heavy metals which have been dumped into the oceans (DeGregori *et al*., 1996).

According to Mateu *et al*. (1996) trace metal levels can be indicators of the concentrations of other pollutants to which they are potentially related.

The quality of the marine environment is constantly being monitored by various national authoritative bodies (such as SEPA in Scotland) analysing water, sediment and/or biota. However, it has been previously demonstrated that a large number of errors may occur owing to the relatively low contents of pollutants (Quevauviller *et al*.,1992).

There is now considerable evidence in the scientific literature that contaminates such as trace metals, phosphorous, pesticides, PCBs and polycyclic aromatic hydrocarbons, can be taken up and concentrated by sediments and suspended matter in aquatic systems. Transportation of these contaminants in association with particulate matter represents a major pathway in the biogeochemical cycling of trace contaminants (Allen, 1979. quoted in Hart, 1982).

Heavy metals belong to the group of elements whose hydro-geochemistry cycles have been greatly accelerated by man. Anthropogenic metals emissions into the atmosphere such as Pb, Hg, Zn, Cd and Cu are 1:3 orders of magnitude higher than natural fluxes. As a consequence these elements are expected to become increasingly accumulated in natural reservoirs. An increase in trace metal concentrations in sea water is not obvious since earlier data on the trace metals concentrations in these systems suffer from inadequacy of sampling technique as well as from a lack of reliable analytical tools (Schindler, 1991).

The results of this study are expected to show that the sediments around the islands in the Archipelago are reasonably unspoiled and that levels of heavy metals are not much higher than the usual background levels for that area. A gradually decreasing profile of pollution is expected to be seen, along the line of the transect, coming from two separated areas: the mouth of the canal and the visibly over-polluted Panama Bay. Since these two sources of pollution are in close proximity to each other, it may be difficult to identify exactly where the pollution is coming from.

The main hypothesis of this project is that the Archipelago is a relatively pristine area and that any contamination or pollution is being carried out from the coast of Panama City on tides and currents.

1.2. AIMS AND OBJECTIVES

- To present a competent and practical baseline review of the concentrations of 10 heavy metals (Zn, Fe, Mn, Pb, Ni, Cu, Cd, Cr, Hg and V) in the marine sediments of the Las Perlas Archipelago, Panama.
- To investigate the source of much of the contamination or pollution within the location.
- To ascertain if there is any correlation between the metal levels and grain size of the samples taken.
- To determine what, if any, detrimental affects these impurities may have on the flora and fauna of the surrounding area.
- To suggest any further studies that could be carried out on the same or similar topics in this area.

1.3. BIOMONITORS

Sediments have frequently been analysed to identify sources of trace metal in the aquatic environment because of the high accumulation rates exhibited (Forstner *et al*., 1981). Sediment analysis allows contaminants that are adsorbed by particulate matter, which escape detection by water analysis, to be identified. The non-residual fraction of the sediment is considered to be mobile and therefore, is likely to become available to aquatic organisms (Waldichuk, 1985).

Biomoniotors are any organisms or systems of the area that can be used to establish variations in the bio availabilities of any paramet6ers, including heavy metals in the marine environment. The use of biomonitors offers time integrated measures of those portions of the ambient metal load that are of direct ecotoxicological relevance (Rainbow, 1995). Other authors prefer to use organisms such as molluscs and marine algae as biomonitors for heavy metals (Szefer *et al*., 1998) since many species of mollusc live within the mangrove ecosystem. It is plausible to use these species as indicators of high levels of pollution within this environment. Concentrations of heavy metals in sediment usually exceed the levels of the overlying water by 3 to 5 orders of magnitude. With such concentrations Zabetoglou *et al*. (2002. quoted in Defew *et al*., 2004) showed that the bioavailability of even a minute fraction of the total sediment metal assumes considerable importance. This is especially true to burrowing and filter feeding organisms. Questions about sediment toxicology to marine organisms and the associated human risk from food animals harvested from contaminated areas need to be raised. Sediments were considered an important indicator for environmental pollution; they act as permanent or temporary traps for material spread into the environment (DeGregori *et al*., 1996).

Coral skeletal growth parameters can also be considered as potential indicators for heavy metals and several environmental factors such as sea surface temperature, nutrients, and runoff, light and human inputs (Guzman & Jarvis, 1996). Since these organisms are widespread in tropical areas, and are considered by some to be considerably easier and more reliable to analyse, the majority of literature available focuses all heavy metal research around these creatures. Although the pathways incorporation into coral skeletons is still unclear, some favourable progress has been

achieved. Metals in corals have been proven as tracers of pollutants in the marine environment; for example, long-term industrial pollution has been detected by measuring phosphorous, lead and cadmium, and short term assessments of several metals have confirmed the potential of metal analysis. However, few of these studies have developed chronologies for metals long enough to be able to separate industrial inputs from background metal concentrations (Guzman & Jarvis, 1996).
Vanadium (V) was found to be a good indicator for environmental studies due to the fact that the element is unlikely to arise from contamination during sampling and analysis compared to other metals. The authors suggest that anthropogenic vanadium might be swamped by background concentrations in surface seawater. The gradual increase of vanadium into the marine environment of Panama during the last 30 years might be a pointer to oil pollution resulting from refinery operations

Furthermore, metal tolerance in corals has been suggested to explain the higher concentrations of some metals and the lack of physiological response (example; bleaching) in corals living under chronic pollution. On the other hand, a reduction in coral skeletal extension was reported in association with high metal concentrations in coral tissue, but it was considered impossible to separate "cause from effect" in the field. We can speculate that most species might be resistant to some degree of pollution in those habitats. Our poor understanding of the physiological response of coral to metal pollution precludes any further discussion on this matter. The observed increase in vanadium and the decline in growth rates in the study area provide strong circumstantial evidence of a sub lethal effect of oil pollution, but not specifically metal pollution in coastal areas. Ecological supporting evidence also points at the oil industry as a major factor responsible for the degradation of the reefs in this area (Guzman & Jarvis, 1996).

More recently, Veron *et al*. (1993 quoted in Jickells, 1995) have extended this work to show that a six fold decline in atmospheric Pb fluxes over ten years. He suggests that this is resulting from the removal of Pb from petrol over recent years. Studies of dissolved Mn and Al concentrations in surface waters around the North Atlantic suggest that atmospheric inputs control concentrations over a wide area.

1.4. PANAMA HISTORY

Between the start of the 16th century until the mid 17th centaury, Panama was known as "Gold Castilla" due to the large quantities of gold found in various parts of the country. In 1520, mining began for the extraction of this precious metal. The majority of operations were located in areas of high precipitation and evaporation. This was thought to have resulted in high losses of Hg and other heavy metals into the environment. Later in the mid 19th century, agriculture, based mainly on banana and coffee plantations, began in the valleys of the rivers of Caribbean Central America, becoming more extensive early in the last century. These agricultural advancements resulted in a dramatic rise in deforestation and consequently in soil erosion and run-off, which has been estimated in millions of tons annually. Despite the variety of mineral deposits and the potential of copper production, the contribution of mining to GDP was negligible. Mining has never accounted for more than a minute part of Panama's Gross Domestic Product falling to US$2.5 million in 1985 from a peak of US$ 4.1 million in 1982. This production was restricted only to the extraction of limestone, clays and sea salt although the potential existed for much more.

Development of Panama's Pacific coastal zone began in the 1940s and boomed in the 1970s as a result of government spending on infrastructure and housing.
This may have contributed to current levels of many heavy metals. It is very probable that the changing land use, over the years, may have contributed significant quantities of metals into the environment. According to Guzman & Garcia (2003) it is, for these reasons, impossible to estimate the amounts and distinguish between natural and anthropogenic source of these heavy metals using available data.
Industrial development has, however, been uneven in Panama. Between 1965 and 1980 industry grew at an average annual rate of 5.9%. Any foreign investments into the country went into relatively large plants for oil refining, food processing and utilities. The first refinery in Panama, (Refneria Panama S.A Bahia Las Minas), started operation in 1962 (Guzman & Jarvis, 1996).

In the 1970s, several copper deposits were discovered. The largest, located in Cerro Colorado, Chiriqui, would be one of the largest copper mines in the world, if

developed. In the 1970s, ore reserves at Cerro Colorado were estimated at nearly 1.4 billion tons. Despite this, the cost of developing the mines, in the late 1970's, was estimated at US$1.5 billion. Commercial exploitation was postponed because of low copper prices on the world market but could be undertaken if copper prices rose substantially in the future.

If the copper mines were to ever be exploited the results could be potentially disastrous for the surrounding. Copper would not be the only metal to be released as many more would be used during the processing and refineries stages.

The world famous Panama Canal does not account for all the oil moving through Panama. The trans-isthmian Oil Pipeline was built to take the excess oil across the country which could not be accommodated by the canal. The pipeline, completed in October 1982, is 81 km long and has a capacity to move 850,000 barrels of oil a day. The pipeline joins two terminals owned by Petroterminales de Panama. It was a joint venture between the Panamanian government and a United States company, Northville Industries. In 1982 the pipeline generated US$69 million, a figure that rose to US$138.8 million in 1986. Externally the rise of oil prices, recession in the industrialised countries and uncertainty relating to the future status of the canal clouded the investment climate.

Between 1980 and 1985, that rate of industrial development had fallen to a record level of -2.2% and the country was thought to be in the midst of a recession. The industrial sector, mainly manufacturing (based on the processing of agricultural products) and mining contributed 9.1% to Gross Domestic Product, followed by construction (4.7%) and energy production (3.4%)[1]. Building rates fell dramatically in 1983 to US$ 106.4 million, when the government cut expenditures and rates continued to decline in 1984 (US$ 94.4 million) and 1985 (US$ 93.4 million).

Previously, Central America's main importance from an international energy perspective was as a transit centre for oil shipments via the Panama Canal. In 2003, approximately 444,000 bbl/d of crude oil and petroleum products passed through the Panama Canal, with around 65% of all oil shipments moving south from the Atlantic

[1] http://workall.com/wfb2001/panam/panama_geography.html (27/07/2005)

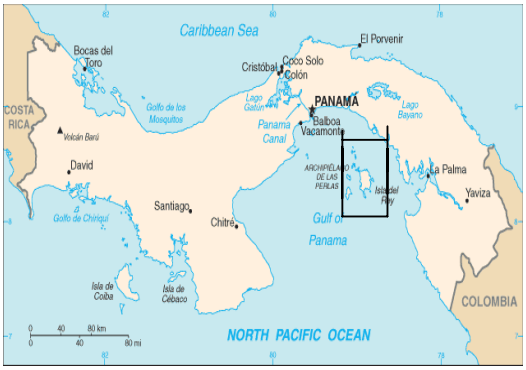
to the Pacific. These figures leave much room for spillage and seepage for example; thought ballast water and dock transfers. Due to the location of the Archipelago De Las Perlas, not far from the Pacific mouth of the canal, the area is left susceptible to some, if not all of the misplaced oil which flows through the canal.

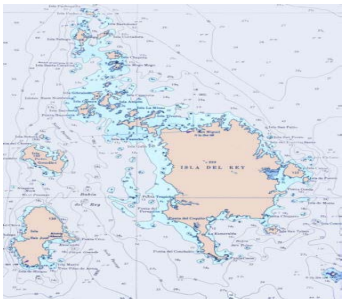
As mentioned before, construction leads to deforestation as more trees and forests are levelled to make way for buildings. This results in an unstable soil structure where the topsoil can easily be eroded. This topsoil, along with local run-off from either agricultural or construction operations ends up in the aquatic environment and can lead to the pollution of that environment by heavy metals. All these possibilities are discussed further in subsequent chapters.

Guzman & Jimenez (1992) have suggested that as a result of the increasing environmental contamination from sewage discharges and oil spills, Central American coastal areas are currently exposed to a larger range of metal pollution (natural and anthropogenic) than ever before.

1.5. SITE DESCRIPTION

The Archipelago of Las Perlas is located approximately an hour and a half from Panama City. The islands are approximately 40 nautical miles in a south-easterly direction from the capital city. The Archipelago of Las Perlas is an amalgamation of over 220 islands, islets and outcrops, only about a dozen of which are inhabited. The main islands are Isla Del Ray (the largest), Contadora, San Miguel, San José and Pedro Gonzalez. Contadora is close to Pacheca, which is an existing protected island of the Archipelago. This gives the tourists and visitors the opportunity to observe a marine life sanctuary and watch birds such as pelicans, boobies and frigates, iguanas, different types of rock crabs and other aspects of marine life. The main industries in the archipelago are deep-sea fishing, and sea angling by tourists. In winter (which runs from May through November) rift lines are formed in the Pacific due to the flush of debris from mainland rivers. This flood brings with it an abundance of nutrients and food for marine life and fishing is plentiful. However, the flush also brings with it an abundance of pollution and run-off from the mainland where deforestation, soil erosion and agricultural practices are much more widespread than on the islands.





This Archipelago is considered to be a relatively pristine area, as it is removed from major human disturbances. The local input of pollution is thought to arise exclusively from residents spilling boat-motor diesel oil or from human or animal effluent. The coastline of the islands are scattered with many small rivers running into the sea. It is thought that fertilizers may be used on a few of the islands for crop growth which may lead to a small amount of run-off into the surrounding ocean. There are a small number of hotels in the Archipelago, mainly on San Jose and Contadora, where there is a 9-hole golf course. Golf course maintenance is a very extensive operation and can cause much run-off into the marine environment which contains an abundance of many different trace and heavy metals.

Sewage and other pollutants are being discharged from the villages into the Pacific Ocean. The runoff of fertilizers from agricultural land is adding to the risk of algal blooms in the region's coastal waters.

The area of the South Pacific is a region that is greatly subjected to excessive current and weather patterns. Even though the Archipelago is surrounded and contained in the Gulf of Panama, currents and weather patterns can still have a strong influence on the dispersion of pollutants from the Panamanian coastline to the islands and visa versa.

The Pacific Central-American Large Marine Ecosystem is a tropical climate and upwelling system that extends along the Pacific Coast of Central America. The continental shelf in that region is narrow and steep and extreme ocean depths are reached very near the coast. The LME is enriched by a high level of nutrients and it carries with it much a warmer upper ocean layer, with a mean ocean temperature of 26°C all year. In general, increased population pressures on the Pacific coast have led to the pollution of rivers, streams, lakes and coastal waters. The lack of adequate facilities for proper waste disposal in the area of Las Perlas creates a variety of pollution situations in the surrounding waters. Pollution from the land is potentially more damaging in the coastal waters of the Northeast Pacific because of the numerous sheltered bays and gulfs where the chemicals cannot easily be dispersed.

The Pacific entrance into the Panama Canal lies within the pathway of the LME. This entrance is only 40 nautical miles from the islands. There is very heavy traffic on these shipping lanes and maritime routes constantly travel in and out of the Gulf past the islands and then follow the entire length of the coastline. This heavy use increases the dangers of marine debris and oil spills. It the danger of these oil spills that have the potential to dramatically increase the metal levels in this area.

Another large natural phenomenon which has a great affect on tropical locations is El Nino. El Nino's warm waters in the Pacific Ocean have caused coral bleaching in the waters off the Pacific coast of Panama.

The exceptional diversity found in Las Perlas suffers high variations in climatic conditions, regular hurricanes and El Nino disturbances that bring both high and low extremes in rainfall where dry and rainy seasons are already extreme. Thus, extreme values are more important than the mean values of river discharge to get an

understanding of the effects of seasonal rainfall on river load and its influence of the subsequent run-off. In this area the river basins are of high relief and are prone to erosion caused by agricultural practices, deforestation, together with steep slopes, seasonally intense rainfall, and high concentrations of people and livestock; the direct result of which is high sedimentation and nutrient loading. Since these islands are mostly uninhabited, there are presently no or little agricultural practices, livestock grazing and deforestation by man. However, this situation can easily change if the area becomes inundated by large tourist resorts, golf courses and agricultural development. It is possible that Las Perlas, like other locations with sever weather conditions, has highly erodible soils which in heavy rainfall will run-off the land into the surrounding sea. This is purely a natural process but the interference of development on the islands could accelerate the outcome. The affects of run-off and atmospheric as well as anthropogenic inputs in this region is dealt with in a succeeding chapter. So if high levels of heavy metals, corresponding to contamination, are found in this area, it can be deduced that they must come from either the mainland or atmospheric inputs.

2.1. CASE STUDIES

Much work has been done on the topic of heavy metals contamination in tropical locations Defew *et al*. (2004) and Guzman & Garcia (2002) in Panama, Guzman & Jimenez (1992) in Panama and Costa Rica, Perdomo *et al*. (1998) in Colombia, DeGregori *et al*. (1996) in Chile and Harris & Santos (2000) in Brazil. However, most of these authors used mangrove sediments to illustrate contamination levels in these locations. This study is slightly different due to the fact that the sediments used are not predominantly mangrove in origin. Given that the sampling location is a tropical location and the abundance of mangrove forests close by, some of the near-shore samples were taken in close proximity to the forests. Most of the samples were taken further off-shore in deeper waters. There has been little work done on non-mangrove sediments in the tropics. There is however, much work available on heavy metal loads in non-tropical locations. This section gives a critical review of all the literature available on this field of study.

Trace metals play an important role in the natural biological life cycles. Their levels help to define the behavior and well-being of individual biological systems and can establish the overall character of a water system. Understanding of this role involves appreciation of the mechanisms of transformation of those trace metals in the system. These considerations relate to both the metals that are essential to biological functions and the metals that may be inhibitory to organisms in the aquatic system. Biological transformations may convert inorganic metal forms to organic compounds of much greater toxicities. A large amount of work has been done by many various authors illustrating the issues mentioned in the above paragraph. The following section is a critical review of those works.

Defew *et al*. (2004) conducted a study similar to this. They used mangrove sediments and leaves to assess the level of heavy metal contamination in Punta Mala (one of the sites involved in this study). Results show that Fe, Zn and Pb were in concentrations high enough to concluded moderate to serious contamination within the bay. Heavy metals cannot be degraded biologically and they are typically transferred and

concentrated into plant tissues from soils. There they pose more long-term damaging effects on plants. If this processes has occurred in this region it is uncertain if the same levels of heavy metals in the sediments will be observed in this study. However, due to the time difference between the two studies, recent atmospheric and anthropogenic deposits may have increased levels even more. The authors have suggested that Punta Mala Bay is accumulating elevated levels of heavy metals, as the bay has semi-diurnal tides as well as high levels of storm water run off from the busy dual carriageway. A number of drains have recently been built during the construction of a major roadway near Punta Mala Bay, which was finished in 1999, and discharges any run off directly into the bay.

According to MacFarlane *et al*. (2003) the majority of studies show few correlations between metals levels in the sediment and metals in tissues, which suggest that mangroves actively avoid metal uptake and / or most metals are present below the sediment bio availability threshold. But this study found sediment metals in concentrations significantly above expected natural background levels and in some cases reaching levels that might be classified as highly contaminated. The authors found that strong linear relationships existed for all metals in root tissue of mangroves. The authors also found that increasing concentrations of Pb and Zn in sediments resulted in a greater accumulation of Pb to both root and leaf tissue.

Belzile *et al*. (2004) undertook a project to investigate trace elements from the Lakes of Killarney Provincial Park in Sudbury, Ontario. These lakes are severely affected by acidification and atmospheric pollutants. They obtained detailed profiles of acid-recoverable trace elements after aqua regia digestion and ICP-OES analysis of sediment cores taken from these lakes. They found that this area is in transformation from a dominant influence of regional pollution sources to combining controlled by continental atmospheric deposition. This was discovered by collecting vertical cores of sediment and distinguishing between the different strata. Vertical sections of sediments have been shown to give detailed records of the historical level of contamination over time. Provided that the pollutants are persistent and the sediment stratum has not been seriously disturbed, a very accurate account can be obtained (Fung, 1993 quoted in Ong Che, 1999).

Coale & Bruland (1987, quoted in Jickells, 1995) found that the natural seasonal concentration cycles and the subsequent stratification of the sediments observed are inevitably complex because the surface mixed layer does not function as a simple closed stastic box. Instead it varies from depths at which atmospheric inputs accumulate until they are mixed out or removed. These results suggest that in relatively undisturbed waters, the top layer of sediment will illustrate the most recent atmospheric and anthropogenic deposits of heavy metals in that region. Schlinder (1991) produced a study to construct a steady state model together with a surface complexation model attempts to give an outline of the factors that control the fate of trace metals in natural aquatic systems and in soil solutions.
Ong Che (1999) suggested that metal concentrations in the upper 0-10 cm of the sediment cores from the mudflat were 4-25% higher than those found in the bottom 21-30 cm.

However, some removal will occur via sinking particles even from a highly stratified system, through probably at a slower rate than from less stratified systems.
Many previous studies of trace metals in natural waters have established the strong enrichment of metals is suspended matter. The importance of this material as a transport medium as a removal mechanism has been stressed by many authors including Wollast (1982) and Harbison (1986).

Most studies show few significant correlations between metal levels in sediment and metals in tissues (MacFarlane *et al*., 2003). These data suggest that either mangroves avoid metal uptake and /or most metals are present with low sediment bio-availability. This clearly indicates that mangroves actively avoid the uptake of trace metals, even when the soil concentrations are high. Zn was found to be the most mobile metal, then Cu, with Pb showing the lowest accumulation to leaf tissue.

Tam & Wong (1999) attempted to classify 18 mangrove swamps in Hong Kong according to their metal contamination according to the grain size. They found that higher concentrations of heavy metals were found in the fine-grained than the sand-sized fractions of the sediment; however, the differences between these two fractions

became less significant when the swamp become more contaminated. These results suggest that as the metal load of contamination in a water body increases, the less selective the metals become concerning binding sites. Due to the sheer volume of literature on the subject of heavy metals and grain size and the on-going debates, an entire section is devoted to exploring both sides of the argument.

Guzman & Jimenez (1992) conducted a survey of 12 metals in the skeletons of coral reef sediments along the Caribbean coast of Coasta Rica and Panama. They indicate high levels of pollution in the region. They suggest that the entire coastline is influenced by hundreds of rivers increasingly loaded with suspended sediments (associated with deforestation) which carry most of the metals several kilometres to the sources at sea. A major source of metals into the marine environment often comes from mining activities. Several cases in Panama have showed the destructive consequences of industrial mining development effluents. The Project of Cerro Petaquilla of Colon will result in the loss of at least 2,500 hectares of forests to give way to the infrastructure for the mine itself and for the roads that will allow access to the area. Although this region is on the northern (Caribbean) coast of Panama and the study site is on the southern (Pacific) coast and, at the time of press, there are no plans of a similar development in the area, this is a good example of the release of heavy metals into the marine environment in a tropical location. (Defew *et al*, 2004).

Perdomo *et al*. (1998) showed that all metals analysed in the tropical sediment of Santa Marta, Colombia were comparable with non-residual concentrations (As, Cd, Cr, Fe, Mn, Ni, Pb and Zn) found in other tropical areas receiving low to moderate contributions of pollution, strongly suggesting an input of industrial discharges.

Fisher & Hook (2002, quoted in Defew *et al*., 2004) showed that when marine copepods were exposed to a variety of metals through their diet, the reproductive capacity decreased by up to 75% because fewer eggs were produced and the hatching success was diminished. These sub-lethal effects occurred at metal concentrations just 2-3 orders of magnitude below acutely toxic concentrations. Exposure of crabs to water-borne copper (Cu) resulted in their reduced ability to osmoregule. The same was found in panaeids while cadmium has been found to elevate haemolymph ions in

crabs. Water-borne copper (Cu) and zinc (Zn) interfere with the respiratory functioning of the gill of crustaceans, resulting in reduces gas-transfer efficiency, a decrease in respitory performance and structural gill damage. Reductions in oxygen consumption and cardiac rate have also been observed following acute exposure to water-borne copper (Cu) (Various authors quoted in Harris & Santos, 2000).

A few studies have shown that these processes occur in nature. It is clear that animals living in chronically polluted area must make adjustments in their regulatory processes to counteract the effects of heavy metals in order to maintain homeostasis and, hence, fitness. There is some evidence that some physiological systems develop "resistance" to heavy metals. They maintain homeostasis in the presence of toxicant concentrations which, normally in "clean"-site populations would inhibit function (Harris & Santos, 2000).

Respiratory impairment is known to occur after exposure to high concentrations of water-borne heavy metals (Spicer & Weber 1992, quoted in Harris & Santos 2000). The evidence presented here suggests that the decapod crab macrofauna, known to be important in energy transfer in mangrove communities and to have a major biopertubation effect on the sediments, accumulates heavy metals in their tissues (Warren & Underwood, 1986 quoted in Harris & Santos, 2000). This may be indicative of reduced fitness, possibly leading to reduced growth rate and lower fecundity.

At the end of the loading period, total plant biomass decreased with increases in wastewater strengths, indicating that high concentrations of heavy metals reduced plant production, especially leaves and roots. It has been reported that the absorbed heavy metals reduced the chlorophyll formation and caused the shedding of the leaves (Siedlecka, 1995). Yim & Tam (1999) also found that heavy metals such as Cd could interfere with the uptake of various nutrient elements, decrease root respiration and inhibit root production

In Austria, total atmosphere deposition has been estimated via analyses of moss samples (Zechmeister 1993, quoted in Sager, 1997). Which were sampled at presumably non-contaminated sites. Pb, Cd, and S-load were significantly correlated with amount of wet deposition, whereas this was not the case for As, Ni and Fe. On

the whole contamination of soils from fertilizers was low with respect to annual input from the atmosphere.

Jickells (1995) produced a review which attempts to summarise our current understanding of the magnitude and effects of atmospheric inputs to the ocean and highlight important areas of uncertainty. Firstly the author considers the evidence that atmospheric inputs affect the chemistry of the oceans. After establishing the significance of the inputs, the reviews continues to consider two important areas of uncertainty in describing the role atmospheric inputs play in global biogeochemistry. The complexities of the atmospheric deposition processes are considered in the third section and the effects of atmospheric deposition on ocean productivity are considered in the final section focussing on the roles of iron and nitrate

Recent studies of coral skeletons reveal the history of surface ocean dissolved Cd and Pb concentrations to be very similar to the predicted atmospheric concentrations with time offsets of a year or less. This is all the more impressive for Cd because of the involvement of this element in rapid nutrient-like cycling in ocean waters (Bruland, 1983 quoted in Jickells, 1995).

Gambrell (1994) is another review paper focusing on the processes affecting the mobility and plant availability of trace and toxic metals in wetlands. In this review the author considers;

1) The release of metals to surface water from sediments and flooded soils.
2) Metal uptake by wetland plants
3) Metal accumulation by benthic and wetland animals
4) Runoff losses
5) Lleaching losses

He suggests that while studying wetland soil processes it is useful to compare wetland and upland soils to appreciate the differences in wetlands. This was the approach taken in this review of trace and toxic metals in wetlands.

Laboratory, greenhouse and field studies have shown that trace and toxic metals are more strongly immobilized under wetland compared to upland soil conditions. Additional research should be done on factors affecting metal uptake under these conditions. Gambrell (1994) suggests that the aspect that should receive more

research attention is how the interaction between soil redox conditions and soil pH affect metal chemistry.

It has been established (Mateu *et al*., 1994) that background levels of trace metals in airborne particle in the North Western Mediterranean region are primarily determined by two factors Saharan dust inputs and isolated pollution events

This type of pollution pattern exists in most parts of the world. There is usually a reliable regional input which creates contamination levels and isolated individual events which enhance these levels up to pollution standards.

Saharan dust is a preface example of long-distance transport of naturally occurring materials. On the other hand, isolated pollution events are of anthropogenic origin

Guzman & Jimenez (1992) provide, for Central America, an assessment of concentrations of several heavy metals over a large geographical area, recorded from coral reefs. They identify potential sources of contamination in the region and discuss implications of continued inputs of heavy metals.

Even the pristine reefs of this region are influenced by metal pollution, although at lower levels than the other reefs. This suggests that a wide range of pollution sources (natural and anthropogenic) and a very effective mechanism for distributing metals are probably influencing the entire region. Almost all heavy metals reported in this study are normal components of fertilizers, lime and pesticides. However, it was not possible to identify source areas or "hot spots" in the coastal areas of Central America.

The objective of Long's (1992) approach was to determine the ranges in chemical concentrations in sediments associated with toxic effects. In other words the authors attempted to ascertain if concentrations ranges in sediments are consistent with the observed effects.

Photosynthesis by diatoms, green algae and sea-grasses also contributes to the precipitation of metal carbonates and hydroxides from water covering tidal mudflats. The rapid removal of carbon dioxide from shallow waster during the day can raise pH by one or two units facilitating carbonate precipitation. Iron and manganese hydroxides, also found under oxidising conditions, will simultaneously remove other

metals from the water body by adsorption and co-precipitation (Hart 1982). Sediment / water interactions on tidal mudflats may also result in the remobilisation of metals from the sediment surface. The chemical environment in shallow water is subject to diurnal fluctuations, particularly during extended periods of slack water. Generally, the highest levels of pH are recorded near midday and reducing conditions prevail in sheltered waters before dawn (Harbison, 1986). Extreme variations in pH could also alter the concentration of dissolved metals and increase their availability to marine organisms.

The additional review addresses three of the possible mechanisms by which trace metals can be concentrated by sediments and suspended particulate matter (Hart 1982). These are physico-chemical adsorption from the water column, biological uptake particularly by bacteria and algae and the sedimentation and physical entrapment of enriched particulate matter. The relative importance of these three mechanisms will be different depending upon the water body involved. There are insufficient studies to allow the establishment of "standard" guidelines about the quantitative importance of the behaviour of heavy metals under different conditions. The importance of natural and natural organic matter in the cycling of trace metals in aquatic systems has been stressed by many authors. This organic matter may complex with the trace metals and keep them in solution, or it may enhance the association of the trace metals with particulate matter by becoming adsorbed to the particulate surface and then complexing with the trace metals in the solution phase. The behaviour of natural organic matter may be the single most important influence on trace metal cycling in aquatic systems and should receive considerably more attention in the future (Hart 1982).

A number of laboratory studies have been reported in which the uptake of trace metals by real sediments was studied. Algae can also concentrate trace metals. For example in a metal polluted stream in Missouri, Hassett *et al*. (1980) reported very high concentrations of lead, zinc and copper in algal samples. They undertook a number of metal uptake experiments and found that metal uptake was dependant upon the metal, the algal species and the pH of the water (Hart 1982). Davies & Sleep (1980) also found that the growth rate, resulting from trace metal uptake in phytoplankton, is more correlated with the amount of trace metal in the phytoplankton

than with the levels in the water (Hart 1982).

International guidelines for pollution classification of sediments are based on the determination of total trace metal concentrations (total decomposition method using strong acids) (Loring & Rantala, 1992, quoted in Perdomo *et al.* 1998)

2.2. POSSIBLE INPUTS

Most of the pollution by heavy metals began with the industrial revolution at the end of the 19th century. As a consequence the fluxes of many trace elements from terrestrial and atmospheric sources to the aquatic environment have increased significantly.

After entering the aquatic environment, trace metals are distributed among water, biotic and sediment compartments. Sediment distribution depends on the physical, chemical and biological properties of the sediments.

1. Non-point source input of pollutants by atmospheric transport (e.g. domestic and industrial sewage, agriculture activities and soil erosion)
2. Point sources of pollutants introduced by rivers and streams
3. A natural change in the mineralogy of the sediments with a relative increase or decrease in the trace metals.
4. Unpredictable point sources (e.g. waste at sea by oil tankers major oil spills)

Heavy metals are natural components of the Earth's crust. The atmosphere is a very dynamic compartment of the earths system and the concentrations of reactive gases and particulate matter are highly variable in space. This variability is the cause of short term day to day changes in aerosols and atmospheric transport and removal processes. An example of long term changes is the massive changes in loadings over the whole Pacific Ocean (Prospero *et al*, 1989), a result of seasonal dust storms in Asia. Whelpdale & Moody (1990) have noted the meteorological intricacy of the coastal zone and the possibility that this may drive complex chemical cycles is explored by Jickells (1995).

For the trace metals, at least, the high-temperature combustion processes which leads to atmospheric emissions results in metal enrichments of particles (Church *et al*, 1990). Enhancement of the long range transport of these elements follows and reduces the efficiency of the deposition processes. Long-distance transport has a marked effect on the temporal variation of trace metal concentrations in aerosols. This type of transport includes isolated pollution events as explained by Mateu *et al.* (1994).
Atmospheric input into the ocean can be by wet or dry deposition and the relative importance of these varies from place to place (depending predominately on rainfall frequency) and from element to element. In general wet deposition is more important than dry for components associated with smaller particles, which are mainly those produced by gas. Such particles, therefore, include many elements and chemical species whose atmospheric sources are dominated by anthropogenic sources (Church *et al*, 1990). Crustal and marine-derived aerosols are characteristically associated with larger aerosols and are more efficiently dry deposited (Jickells, 1995). Atmospheric inputs deposit directly into the oceanic euphotic zone. Fluvial inputs are subject to considerable modification by biogeochemical processes in estuarine and coastal waters before they mix with the ocean in a variety of complex and, according to Jickells (1995), poorly understood ways.

Trace metals and nutrients are largely transported in the atmosphere as aerosols, with the exception of Hg compounds which travel mainly in a gaseous state, which is rather inefficiently deposited. The estimates of atmospheric inputs of metals to the oceans and some coastal areas are reviewed and the uncertainties in these estimates considered in Jickells (1995). However, as suggested by the author, there are still major uncertainties in the understanding of the interactions between the atmosphere and the ocean for these elements.

In previous years gasoline burning was predominately the major source of atmospheric lead but in recent years has declined due to the use of unleaded petrols. Primary industrial production of lead is largely responsible for approximately 60% of the atmospheric copper.

Recently atmospheric inputs have attracted a more general interest over the question of Fe inputs to the ocean. Martin's proposal (Martin 1990) of Fe limitation of primary production in some areas of the ocean has caused considerably controversy.
Therefore, though transport and settling, heavy metals may pose a threat to the aquatic environment. In particular, solids attract toxic metals, i.e. Zn, Pb, Cu and Cd, which may be released into the dissolved phase. Biogeochemical processes in surface seawater (e.g. particle passage through zooplankton guts) may solubilise the particulate matter (Jickells, 1995). Metal species produced as a result of the phyto-plankton decomposition will re-equilibrate to replace the free metal ions lost and could then cycle again. The only major difference from the lake cycle is the inclusion of a benthic algal reservoir (Bjerkelund, 1981). Allen (1979) has pointed out the particulate matter is an extremely important and, to date poorly studied, substrate for the transportation of trace metals in fluvial systems (Hart, 1982).

Rivers appear to be the most important sources of heavy metals into the sea as they carry much larger quantities of elements as particulates than they do as solutes (Bryan, 1976). All rivers in Central America are characterised by a high suspended sediment concentration as a result of deforestation and soil erosion. During the rainy season (8 months April – Nov), run off increases and the amount of suspended matter can be above 10mg 1^{-1} (Guzman *et al*., 1991). Most of these materials are clays which bind metals easily.

Natural and anthropogenic metals are transported from land to the sea mainly through rivers and the atmosphere. In the marine environment the land derived detrital metals, along with the non-detrital metals which have been removed from the water column, settle towards the bottom and form marine sediments (Angelidis & Aloupi 1997). The same general processes occur in the littoral environment. However, the local runoff material plays the key role in the morphology and mineralogy of the coastal sediments. This material is directly influenced by the local geology, as well as, the local human metal sources (industrial and urban effluents).
Part of this particulate matter will be lost to the sediments by sedimentation, although it may also stay in suspension long enough to take up more metals).

Surface runoff is a potential process by which metals (and other forms of pollution) may be removed from contaminated wetland and upland soils. If contaminated materials are placed in locations where drainage does occur, then elevated losses of Cu, Ni, Zn, and Mn will occur but according to Gambrell (1994), Cr losses do not follow this trend. He suggests that chromium tends to remain in upland soils rather than wetland soils. Most urban and industrial runoff contains a component of trace metals in dissolved or particulate form (Hart 1982) which rapidly decreases in concentration with distance from the outfall.

Flux from fertilizers is influenced by the local farming practice, as well as from fertilizer production. Flux from total deposition, however, cannot be influenced by the farmer because it derives from atmospheric pollution. Secondly, input via fertilizers occur discontinuously, whereas input via deposition is continuous (Sager, 1997). Cu, Zn and Mo are essential for plant growth and thus sometimes artificially added to many fertilisers.

In general, point-sources can be identified and actions can be taken to manage and mitigate the problem. However, in Central America, point source effluents are generally discharged into common areas and it is not always possible to identify or assess the specific effects of contaminants on the environment. For more than 20 years, pesticides have been used intensively and indiscriminately in Central America, introducing hundreds of tons of chemicals into the environment (Guzman & Jimenez, 1992). Discharge of oil at sea, the use of anti-fowling and anti-corrosive paints, oil spills during shipping and terminal transfers and effluent discharges from refineries are probably among the anthropogenic sources of Pb, Cr, Fe, Cu, Zn, Cd and V into the oceans. Major ecological impacts have also developed in remote areas far from shore.

Other major anthropogenic sources of atmospheric trace elements include high temperature processes in steel and iron manufacturing, cement production and in a number of small plants where metals are applied in the production process. When emissions are calculated special emphasis should be placed on the technology

employed in the plants. Large differences can usually be observed between the emission factors for various production technologies due to the reuse of trace element containing scrap. Generally waste incineration is gaining much interest due to the emissions of Cd, Hg, Pb, Sb and other trace elements due to a need to incinerate an increasing amount of wastes. Trace elements emissions from municipal incineration depend on the proportion of combustible and non-combustible material in the refuse input, the chemical composition of the input, the incinerator design and the efficiency of control devices. As these factors may vary from one country to another, the application of emission factors is very limited. (*Pacyna et al*., 1991).

Traditionally budgets and residence time estimates for trace metals and nutrients in the ocean have been based on river fluxes and have ignored atmospheric and other inputs. There area a few exceptions to this generalisation, perhaps most notably for clay minerals in ocean sediments. However, for most major and minor components of seawater the atmosphere has generally been assumed to be a secondary source. Over the last 20 years or so this view has been modified and systematic studies of atmospheric inputs to the oceans have been undertaken. Sufficient data are now available to allow fairly reliable estimations to be made of the atmospheric inputs to the oceans (Jickells, 1995).

Since sediments often constitute the ultimate depository environment for trace elements introduced into aquatic systems, the solid-phase distribution can reflect the history of pollution assuming that those metals are not mobilised substantially following deposition (Belzile et al 2003).

The destruction of forest areas ultimately results in an increase in soil erosion and higher input of both natural and anthropogenic sources of metals. In addition, the region is affected by trace metals released into the environmental from extensive deforestation, agricultural practices, topsoil erosion and runoff excesses of fertilisers and agrochemicals (Guzman & Jiminez, 2002).

As a consequence, in order to evaluate patterns of metal concentrations in coastal marine sediments, these data have to be examined while taking into consideration i)

grain size variability, ii) the geo-chemistry of the local material, iii) the effect of the human discharges into the marine environment (Angelidis & Aloupi, 1997).

2.3. INFLUENCING FACTORS

Comparison of total trace metal content in sediments in different areas may be a convenient way of expressing some measure of pollution, but this method has its limitations. Sediment metal concentrations are influenced by a range of factors. They include physical and hydrological characteristics of the region and its benthos, atmospheric conditions, productivity, pH, soil texture, redox potential and cation exchange capacity among others. Belzile *et al*. (2003) suggested that the internal geochemical processes that could lead to the remobilization of pollutants such as certain trace metals should be taken into account also.

The quantity of heavy metals retained in sediments is also affected by the characteristics of the sediment into which they are adsorbed. Grain size, partition co-efficient (Kd), cation exchange, organic matter content and mineral constituents all influence the uptake of heavy metals in the aquatic environment. However, unlike many other authors, Gambrell (1994) suggests that elevated concentrations of metals do not necessarily pose a threat as they may never be released from the sediments and therefore may not be available for excessive plant uptake.

It is well known that sediments reflect an area's productivity. Although as Schindler *et al*. (1996, quoted in Belzile *et al*., 2003) suggested, a significant change in water clarity can alter the biological processes of deep water and littoral environments. The specific fate of heavy metals in the environment cannot be completely understood due to unexpected acts of nature. However, the best alternative is to make educated estimations using the available data.

All soils and sediments contain some concentration (usually low) of trace and toxic metals from natural sources. However, these background levels can vary widely depending on a number of factors such as parent material, sedimentation processes in water bodies and other things. It is usually a result of human activities that levels of

metals increase and due to this pollution in soils and sediments can rise to the point where they represent a potential health or ecological risk.

Water soluble metals are the most mobile of all the heavy metals and are also the most readily available for plant uptake. Exchangeable metals, however, are those that rather than existing in the water column, are primarily bound to soil surfaces by cation exchange processes. Metals that are found in this form are considered to be bonded very weakly and may be displaced easily to the water-soluble form. Together, the metals in the soluble and exchangeable form are considered readily mobilized. When changes occur in the oxidation status of soils and sediments, transformations of metals between chemical forms, soluble and insoluble, may occur. This affects the mobility and plant availability of metals.

Soil oxidation conditions also influence soil pH, a major factor influencing metal chemistry (Gambrell, 1994). Schlinder (1991) suggests that the pH value of the solution is the master variable that oversees the adsorption of metal ions at surfaces. High pH values promote adsorption whereas low pH can actually prevent the retention of metals by sediment (Belzile *et al*., 2003).

Stumm & Morgan (1981) have suggested that pH can not only markedly affect the type surface sites and but also the speciation of the metal ion in solution adsorbed out of solution. The results of Gambrell (1994) support this idea and indicate that permanently flooded sediment becomes strongly acidic (i.e. reduced with a low pH) upon drainage, the process which retains metals tends to be intensified. These finding propose that large-scale metal releases do not occur with changing redox conditions. This is in contrary to the previous idea, by many authors that redox reactions affect the uptake and release of heavy metals from sediments.

Areas that lack tidal flushing and good water circulation exhibit anaerobic conditions and tend to favour the formation of metal sulphides. This is due to the action of sulphate reducing bacteria which implements high pH values in the sub-surface sediments. Precipitation of metals at the sediment-water interface is encouraged by these high pH values and this all contributes to the retention of metals as sulphides.

Alternatively, the environment (especially mud) subject to a periodic immersion and emersion regime is a more aerobic environment, especially at the sediment-water interface. According to Ong Che (1999) these are the areas in which metal attachment is maximum. Furthermore, because of the aeration of the upper layers of the sediment (due to the oxidation of sulphide by bacteria) the metals are boundary-less and can mobilize and be exported to deeper waters.

In deeper water, however, incorporated trace metals are transported back to the surface by up welling. In areas of constant upwelling, i.e. in the deeper waters of the Atlantic and the Pacific, heavy metals are thought to be almost constantly in suspension and circulation due to upwelling cycles. There is also the threat that, due to current patterns, these pollutants are returned to the coastal zone. According to Li (1981) transport by biota and biological debris is important for the lower part of the water column. Mackey and Hodgkinson 1995 (quoted in Ong Che, 1999) suggested that metal concentrations tended to increase from land to sea. The authors attributed this to the role of tidal deposition in determining the spatial distribution of metals in deep water sediments. Many authors suggest that it is in the open sea where these pollutants can cause most harm. Sigg (1987) indicated that the adsorption and / or uptake by biota is even more pronounced in the open ocean, in isolated locations that are at sites near pollution sources.

Schindler (1991) has commented on the work of Bruland (1980) when he assessed the levels of (some) heavy metals in association with nutrients in the North Pacific. Schlinder suggests that the profiles that Bruland produced indicate that aquatic micro organisms in the surface of the open ocean control the concentrations of many of the trace organisms. He does not elaborate on this statement in his paper or explain how this process occurs.

Recently a large discussion over the role of climate change in pollution has arisen over the past few years. Many scientists seem to believe that there is a link between sea warming and metal loads in the environment, especially levels found in marine organisms. Rainbow (1990) has indicated that heavy metals and many other

pollutants are expected to be absorbed more rapidly at higher temperatures. Climate change is also considered to be capable of altering the productivity and the biological and chemical recovery of stressed environments. This may also affect certain sediments, which in previous episodes of pollution, has been capable of self restoration.

Tam & Wong (2000) found that higher concentrations of heavy metals were found in the fine-grained fraction of the sediment rather than the sand-sized fractions. The difference, however, became less significant when the region became more contaminated / polluted. Heavy metals arriving on the incoming tide or entering from fresh water sources were rapidly removed from the water and deposited onto the sediments. These results suggest that the source of the pollution is irrelevant as the heavy metals are instantaneously adsorbed into the sediments.

There is much literature available on the varying ability of heavy metals to adhere to different grain sizes. The affinity of a metal to associate with particulate matter is described by its partition co-efficient (Kd). It has been shown, by many authors, that large amounts of heavy metals are bound in the fine grain fraction (< 63 um) of the sediment: mainly because of its high surface are to grain size ratio and humic substances content (Tam & Wong, 2000). A subsequent section in this study has been devoted to this topic and it will be discussed in detail.

The Kd for a metal varies in response to changes in salinity and the nature of the particulate matter present. Mercury and lead have higher coefficients than other metals. Such metals are absorbed onto sediments have a much greater retention time and tend to remain in the dissolved phase, such as cadmium.

2.4. METALS IN SEDIMENTS

Natural background levels of heavy metals exist in the majority of sediments due to mineral weathering and natural soil erosion. It is when man's activities accelerate or antagonise these processes that the background levels are increased, by pollution, to levels that have detrimental effects on the environment. Sediments with low heavy metal concentrations are not necessarily "natural" just because the levels are indeed low. They may represent a mixture of small quantity of pollutants diluted by a large amount of natural sediment with low heavy metal content. (Herut *et al*, 1993).

In the past sediments and particulate matter have been considered as purely abiotic material. This is obviously not the case and it is now well known that sediments contain large bacterial populations. Sediments are also complex mixtures of a number of solid phases that may include clays, silica, organic matter, carbonates and large bacterial populations.

There are three possible mechanisms by which trace metals may be taken up by sediments and suspended matter

1) physicochemical adsorption from the water column
2) biological uptake by organic matter or organisms
3) physical accumulation of metal enriched particulate matter by sedimentation or entrainment

Physicochemical adsorption direct from the water column happens in many different ways. Physical adsorption usually occurs when particulate matter directly adsorb heavy metals straight from the water. Chemical and biological adsorption are more complicated as they are controlled by many factors such as pH and oxidation.

There is a lack of detailed knowledge about the specific nature of sediment surfaces. This is mainly due to the high concentrations used in most adsorption experiments which are unrealistic and would not occur naturally

A number of studies have shown that metal ions are strongly adsorbed by solid organic matter. The structure and composition of humic matter can vary considerably depending upon its origin and can be expected to influence the results of sorption experiments. Natural organic matter has a very important influence on the distribution of trace metals in aquatic systems. In addition uptake may be actively completed by bacteria and algae. This results in sediment enrichment. Sedimentation of enriched particulate matter is the other potentially important mechanism by which sediments may concentrate trace metals (Hart 1982).

There is no evidence to suggest that trace metal binding to solid natural organic matter should be any different to that by soluble natural organic matter. The difference between these surface types is not well understood particularly with respect to trace metal uptake. . Gardner (1974) found that adsorption of cadmium by river mud samples was very rapid (in the order of minutes) and that some additional adsorption occurred over a further 24hour period. Within the soil, trace metals can be either transformed to less soluble forms (as discussed in previous chapters) or they can move to living biota. There is also the possibility that they may be eluted into the watershed and contribute to diffuse pollution in that area.

Elevated levels are helped also by the oxidation of surface sediments due to periodic drying between tides. This, incorporated with some biological processes such as bioturbiation or O_2 release from mangrove roots, can enhance uptake rates. This exposure to O_2 results in the oxidation of sulphides in the sediment. A reduction in sediment pore water pH due to production of sulphuric acid, allows the mobilisation of metals (Clark *et al*., 1998).

Many authors propose that the interface between water and sediment plays many important roles in the chemistry of trace metals. Firstly, the upper layer of sediment is usually oxidised (as previously stated) and therefore, acts as a diffusion barrier for mobilized solutes travelling upward from reducing zones of sediment.
Secondly, the surface sediments on the bed of many estuaries exchange readily with suspended solids in the water column and therefore easily adsorb any passing

material.

Ultimately, Szefer & Geldon (1998) suggest that the sediments at the water interface (i.e. the topsoil) are more important to biological fauna than when compared with subsurface meiofauna. They, therefore, offer a higher opportunity for uptake by benthic organisms.

Long (1992) suggests that the oxidation-reduction potential and the concentration of sulphides in the sediments can strongly influence the concentration of trace metals and their availability. Clark *et al*. (1998) explain that the redox potential of the sediment can affect metal trapping directly through change in the oxidation state of the metal itself. Or indirectly through a change in the oxidation state of the ions that can form complexes with the metal.

Additional loads of pollution, especially those gained from run-off, in surface waters, of nutrients and trace metals derived from soil erosion processes are largely influenced by the kind of crop grown on the surrounding land. Many heavy metals, especially mercury, have a high capacity for long range atmospheric transport or through marine currents by thousands of kilometres in only a few months (Rasmussen, 1998. quoted in Guzman & Garcia, 2003).

Depending upon the environment the sediment particle size distribution may range from very small colloidal particles (of < 0.1um in diameter) to large sand and gravel particles several millimetres in diameter. There is a small variation between the mobility of particulate in river waters and seawater. This is very supervising due to a wide expected variation in particle types. Therefore, metals and the subsequent pollution will progress equally in both rivers and the ocean. Harbison (1986) has reported that tidal mudflats and particularly mangrove substrates contain a much greater load of trace metals than other shoreline sediments. This is where the sediments are most vulnerable to the environmental parameters that might influence the migration of these metals.

Calcium (Cd) and manganese (Mn) ions may also influence the sorption of other trace metals ions. This happens, on oxide surfaces, in either of three ways.

1) Firstly Cd and Mn are normally present at concentrations many orders of magnitude higher than the other trace metals. They may, therefore, occupy most of the surface binding sites and leave little opportunity for binding of other metals even though they form less stable surface complexes.

2) Tipping (1981) showed that twice as much natural fluvial (changeable) heavy metal material was sorbed to goethite (hydrated iron oxide sediments, common in areas of large ore deposits) when calcium and magnesium were present than when absent.

3) Recent work by Benjamin & Leckie (1980 & 1981), however, suggests that oxide surfaces may consist of many groups of binding sites. The strength of binding between a given metal ion and the surface may vary by an order of magnitude, from one site to another. At small sorption densities all types of sites are available in excess. Hart (1982) supports this statement by reporting that at higher adsorption densities the availability of the strongest binding sites decreases in the apparent adsorption equilibrium constant. This seems to occur only when a few percent of all surface sites are occupied.

Vertical sections of sediments can give detailed records of the historical level of contamination over time. Provided that the pollutants are persistent and the sediment stratum has not been seriously disturbed, a very accurate account can be obtained (Fung, 1993 quoted in Ong Che, 1999).

2.5. METALS IN MANGROVES

Mangrove muds possess intrinsic physical and chemical properties and an extraordinary capacity to accumulate materials discharged to the nearshore marine environment according to Harbison (1986). The sheltered and stagnant water environment of mangroves allows extensive sedimentation of the finest clay, silt and detrital particles. This material is bound and stabilized by a tangled mat of root hairs growing horizontally just below the mud surface. These particles provide optimum surfaces for trace metal transport (as will be discussed further in the following chapters). During prolonged periods of standing water, changes in pH may affect the migration of metals at the sediment surface and the concentration of free metal ions in overlying water. Due to the fact that the majority of tropical sediments consist of mangrove mud, these findings would suggest that these sediments are pollution traps as well as nutrient traps.

Although mangrove ecosystems can act as sinks for heavy metals, they can also become pollution sources to plants and soils. The influence of heavy metals on photosynthesis and other physiological processes in plants is quiet well known (Yim & Tam, 1999). In all the literature that has been reviewed, it seems that lead (Pb) has the most detrimental effect on plants and photosynthetic processes. Although according to Koeppe (1981) lead accumulations in localized areas of pollution sources probably have little direct effect on plants. The lack of effect is due to the almost irreversible binding of lead to the soil exchange surfaces. If they do enter the plants the major effect will be to the food chain, the topical lead coating may be ingested by herbivores grazing on these plants.

Mangroves grow at sites which are permanently covered by shallow water or are flooded seasonally usually at the upper tidal level on sheltered coastlines. At tropical latitudes, many species tolerate water-logged, muddy substrates. It is well known that mangrove sediments are under permanently reducing conditions and contain high concentrations of organic matter and sulphides. Thus, these sediments favour the retention of the water-borne heavy metals and are generally not available for uptake

by mangrove plants (Harbison, 1986) and probably by other organisms. This statement supports those of Koeppe (1981) in the above paragraph.
Mangrove muds contain more than 85% particulate sediments in the clay / silt fraction (<63um) and that, irrespective of proximity to the various sources, promote higher trace metal concentrations.

Mangrove roots often act as a barrier, retain most of the heavy metals and reduce the translocation of heavy metals to other plant parts. It has been suggested that Cu is more mobile in mangrove plants than other metals. In general, very small amounts of heavy metals are found in leaf tissues as most absorbed heavy metals are accumulated in the stem and roots (Yim & Tam, 1999).

Microbial degradation of the high content of organic matter in mangrove muds generally removes all oxygen from sediments below the surface layer, creating ideal conditions for bacterial sulphate reduction (Berner, 1983). When photosynthetic oxygen production ceases during the night, H_2S diffuses upwards through the mud and escapes to shallow water covering the sediments (Hansen *et al*., 1978). Metals dissolved in this water as free ions or metal-humate complexes, are deposited as sulphides (Harbison, 1986).

2.6. METAL SPECIATION AND TOXICITY

The term heavy metal may have various general or more specific meanings. According to one definition, the heavy metals are a group of elements between copper and lead on the periodic table of the elements; having atomic weights between 63.546 and 200.590 and specific gravities greater than 4.0. Under this definition only 5 of the metals to be analysed in this study are considered 'heavy'. Copper, zinc, cadmium, mercury and lead are all within the confines of the classification. Living organisms require trace amounts of some heavy metals but excessive levels can be detrimental to the organism. However vanadium, chromium, manganese, iron and nickel are above copper on the periodic table and are all very important due to their effects on organisms. Their accumulation over time in the bodies of mammals can cause serious illness. Even though they are not fundamentally 'heavy' metals, the five metals listed are just as, if not more, detrimental to the environment and more important to this study than those metals which do fit the criteria to be called heavy. It is with this statement in mind that they are included in this study and will, for the duration of this paper, be referred to as 'heavy' metals.

A stricter definition restricts the term to those metals heavier than the rare earth metals, at the bottom of the periodic table. None of these are essential elements in biological systems; all of the more well-known elements with the exception of bismuth and gold are horribly toxic. This definition, however, does not include any of the 10 metals as 'heavy'.

All of these separate metals and their presence in the environment will be discussed in this section. The consequent beneficial or detrimental effects including toxicity and hazards will be also be considered.

Cadmium (Cd)

Cadmium is produced as an inevitable by-product of zinc and occasionally lead refining, neither of which are known to be mined in South America. Cadmium has a chemical similarity to that of zinc, an essential micronutrient for plants, animals and

humans. Cadmium is very biopersistent but has few toxicological properties and once absorbed by an organism, remains resident for many years. The most significant use of cadmium is in nickel / cadmium batteries, as rechargeable or secondary power sources exhibiting high output, long life, low maintenance and high tolerance to physical and electrical stress. Cadmium coatings provide good corrosion resistance coating to vessels and other vehicles, particularly in high stress environments such as marine and aerospace. Other uses of cadmium are as pigments, stabilisers for PVC, in alloys and electronic compounds. Cadmium is also present as an impurity in several products, including phosphate fertilisers, detergents and refined petroleum products.

Chromium (Cr)

Chromium is used in metal alloys and pigments for paints, cement, paper, rubber, and other materials. It has also been found to be an effective anti-fouling component. Regulations are currently being implemented world wide to decrease the use of anti-fouling treatments to prevent the leakage of various metals into the marine environment. Airborne emissions from chemical plants and incineration facilities are some of the most common releases of chromium. They are derived from effluents from chemical plants, contaminated landfill, topsoil and rocks. Chromium often accumulates in aquatic life, adding to the danger of eating fish that may have been exposed to high levels of chromium.

Copper (Cu)

Copper is an essential micro-nutrient required in the growth of both plants and animals. In humans, it helps in the production of blood haemoglobin. In plants, copper is especially important in seed production, disease resistance and regulation of water. Copper is indeed essential, but in high doses it can cause anaemia, liver and kidney damage, and stomach and intestinal irritation. Copper normally occurs in drinking water from copper pipes, as well as from additives designed to control algal growth. While copper's interaction with the environment is complex, research shows that most copper introduced into the environment is, or rapidly becomes, stable and results in a form which does not pose a risk to the environment. In fact, unlike some man-made materials, copper is not magnified in the body nor bio-accumulated in the food chain.

Lead (Pb)

Lead occurs naturally in the environment. However, most lead concentrations that are found in the environment are a result of human activities, natural and anthropogenic sources. Lead is among the most recycled non-ferrous metals and its secondary production has therefore grown steadily in spite of declining lead prices. Its physical and chemical properties are applied in the manufacturing, construction and chemical industries. Lead is most common in batteries and petrol additives which are no longer allowed in the EU but their use is still permitted in Panama. Exposure to lead can result in a wide range of biological effects depending on the level and duration of exposure. Various effects occur over a broad range of doses, with the developing young and infants being more sensitive than adults. Lead poisoning, which is so severe as to cause evident illness, is now very rare. For as is known, lead fulfils no essential function in the human body, it can merely do harm after uptake from food, air or water. Lead is a particularly dangerous chemical, as it can accumulate in individual organisms, but also in entire food chains.

Mercury (Hg)

Mercury is a toxic substance which has no known function in human biochemistry or physiology and does not occur naturally in living organisms. It rarely occurs free in nature and is found mainly in cinnabar ore (HgS) in Spain and Italy

Monomethylmercury is probably the most common toxic form of mercury found in the marine environment. It has been known to travel through marine food chains and causes damage to human consumers. Mercury is a metal that occurs naturally in the environment. It enters the environment as a result of normal breakdown of minerals in rocks and soil through exposure to wind and water. Release of mercury from natural sources has remained fairly the same over the years. Still mercury concentrations in the environment are increasing; this is ascribed to human activity. Most of the mercury released from human activities is released into air, through fossil fuel combustion, mining, smelting and solid waste combustion. Some forms of human activity release mercury directly into soil or water, for instance the application of agricultural fertilizers and industrial wastewater disposal. All mercury that is released in the environment will eventually end up in soils or surface waters.

Vanadium (V)

Vanadium is an abundant element in crude oils and considered to be a toxic element in the environment. It can have an inhibitory effect on some organisms and has been used as a proxy tracer of oil pollution along the Caribbean coast of Panama (Guzman & Jarvis 1996). Background seawater concentrations were observed in the early history of corals followed by an increase after 1962, the initiation of a refinery operation. This chronology suggests that a major degradation process in the Panamanian coastal zone could have started around the 1960's. Due to the location of the Archipelago and its proximity to the mouth of the canal and various shipping channels, the likelihood of high levels of vanadium in the sediments is very probable. Vanadium can be found in the environment in algae, plants, invertebrates, fishes and many other species. In mussels and crabs vanadium strongly bioaccumulates, which can lead to concentrations of about 10^5 to 10^6 times greater than the concentrations that are found in seawater. Vanadium causes the inhibition of certain enzymes in animals, which has several neurological effects. Next to the neurological effects vanadium can cause breathing disorders, paralyses and negative effects on the liver and kidneys. Laboratory tests with test animals have shown, that vanadium can cause harm to the reproductive system of male animals, and that it accumulates in the female placenta. Vanadium can cause DNA alteration in some cases, but it cannot cause cancer in animals.

Nickel (Ni)

Nickel is a compound that occurs in the environment only at very low levels and is essential in small doses but it can be dangerous when the maximum tolerable amounts are exceeded. This can cause various kinds of cancer on different sites within the bodies of animals, mainly of those that live near refineries. The most common application of nickel is an ingredient of steal and other metal products. Nickel is released into the air by power plants and trash incinerators and will settle to the ground or fall down after reactions with precipitation. It usually takes a long time for nickel to be removed from air. Nickel can also end up in surface water when it is a part of wastewater streams. The larger part of all nickel compounds that are released

to the environment will adsorb to sediment or soil particles and become immobile as a result. In acidic ground however, nickel becomes more mobile and will often rinse out to the groundwater. Microrganisms can also suffer from growth decline due to the presence of nickel, but they usually develop resistance to nickel after a while. Nickel is not known to accumulate in plants or animals and as a result nickel has not been found to bio magnify up the food chain. For animals nickel is an essential foodstuff in small amounts.

Manganese (Mn)

Manganese is a very common compound that can be found everywhere on earth. Manganese is one out of three toxic essential trace elements, which means that it is not only necessary for humans to survive, but it is also toxic at high concentrations. Manganese effects occur mainly in the respiratory tract and in the brain.. Manganese compounds exist naturally in the environment as solids in the soils and small particles in the water. Manganese particles in air are present in dust particles. These usually settle to earth within a few days. Humans enhance manganese concentrations in the air by industrial activities and through burning fossil fuels. Manganese that derives from human sources can also enter surface water, groundwater and sewage water. Through the application of manganese pesticides, manganese will enter soils.

For animals manganese is an essential component of over thirty-six enzymes that are used for the carbohydrate, protein and fat metabolism.. In plants manganese ions are transported to the leaves after uptake from soils. When too little manganese can be absorbed from the soil this causes disturbances in plant mechanisms. Manganese can cause both toxicity and deficiency symptoms in plants. When the pH of the soil is low manganese deficiencies are more common.

Iron (Fe)

Iron is the most abundant metal, and is believed to be the tenth most abundant element in the universe. Iron is a metal extracted from iron ore, and is hardly ever found in the free (elemental) state. Iron is the most used of all the metals, comprising 95 percent of all the metal tonnage produced worldwide. Its combination of low cost and high strength make it indispensable, especially in applications like automobiles, the hulls

of large ships, and structural components for buildings. Steel is the best known alloy of iron. Iron is essential to all organisms, except for a few bacteria. It is mostly stably incorporated in the inside of metalloproteins, because in exposed or in free form it causes production of free radicals that are generally toxic to cells. Iron binds avidly to virtually all biomolecules so it will adhere nonspecifically to cell membranes, nucleic acids, proteins etc. Iron distribution is heavily regulated in mammals. The iron absorbed from the duodenum binds to transferrin, and carried by blood it reaches different cells. It is strongly advised not to let the chemical enter into the environment because it persists in the environment. Excess iron in the body causes liver and kidney damage (haemochromatosis). Some iron compounds are suspected carcinogens.

Zinc (Zn)

Zinc occurs naturally in air, water and soil, but zinc concentrations are rising unnaturally, due to addition of zinc through human activities. Most zinc is added during industrial activities, such as mining, coal and waste combustion and steel processing. Zinc is a very common substance that occurs naturally. Many foodstuffs contain certain concentrations of zinc. Drinking water also contains certain amounts of zinc, which may be higher when it is stored in metal tanks. Industrial sources or toxic waste sites may cause the zinc amounts in drinking water to reach levels that can cause health problems. Zinc is a trace element that is essential for human health. Zinc-shortages can cause birth defects. The world's zinc production is still rising which means that more and more zinc ends up in the environment. Water is polluted with zinc, due to the presence of large quantities present in the wastewater of industrial plants. This wastewater is not purified satisfactory. One of the consequences is that rivers are depositing zinc-polluted sludge on their banks. Zinc may also increase the acidity of waters. Some fish can accumulate zinc in their bodies, when they live in zinc-contaminated waterways. When zinc enters the bodies of these fish it is able to bio magnify up the food chain. Water-soluble zinc that is located in soils can contaminate groundwater. Plants often have a zinc uptake that their systems cannot handle, due to the accumulation of zinc in soils. Finally, zinc can interrupt the activity in soils, as it negatively influences the activity of microrganisms and earthworms. The breakdown of organic matter may seriously slow

down because of this.

TOXICITY

Metals have the potential to be toxic to living organisms if present at availability above a threshold level. This threshold varies between taxa and metal speciation. Most urban and industrial runoff contains a component of trace and heavy metals in the dissolved or particulate form (Defew et al. 2004).

Contamination caused by trace metals affects the ocean waters, the contential shelf and the coastal zone, where besides having a longer residence time; metal concentrations are higher due to the input and transport by river runoff and the proximity of industrial and urban zones. The impact of anthropogenic perturbation is most strongly felt by estuarine and coastal environments adjacent to urban areas. Heavy metals from incoming tidal water and fresh water sources are rapidly removed from the water body and are deposited onto the sediments (Guzman & Garcia, 2002).

Since heavy metals cannot be degraded biologically, they are transferred and concentrated into plant tissues from soils and pose long-term damaging effects on plants. Nevertheless, different plants react differently to wastewater irrigation; some are more resistant to heavy metals. The ability of mangrove plants to tolerate heavy metals in wastewater is not clear and the impact of wastewater on plant growth must be understood before the system can be employed for removing heavy metal from wastewater. Heavy metals that accumulate in soils not only exert deleterious effects on plant growth, but also affect the soil microbial communities and soil fertility. Yim & Tam (1999) found that microbial biomass and enzyme activities decreased with increasing heavy metal pollution, but decreases vary depending on the types of enzymes.

The potential hazard to the marine environment of pollutants depends mostly on their concentration and persistence. Persistence pollutants, such as heavy metals, can remain in the environment unchanged for years and thus may pose a threat to man and other organisms. The pollution levels and wide distribution reported here suggest that

heavy metals must be considered a serious regional threat. Inadequate or no sewage treatment, increasing waste from industrial and particularly agricultural activities, oil spill and soil erosion (Thapa & Weber, 1991) are just a few of the chronic problems that Central American countries have faced over the last two decades.

All the problems associated with heavy metal pollution will increase considerably in the years to come if measures for control and management are not created. Metal accumulation in agricultural soils together with associated natural metal erosion will remain a chronic pollution problem in the future. A major regional problem is associated with deforestation, increasing soil erosion from agricultural activities, consequent run-off of both natural and anthropogenic metals and long-distance transport of pollutants from industrial areas. Therefore, the problem extends beyond any local or national borders and must be managed at regional levels (Guzman & Jimenez 1992).

RECENT FINDINGS

Scientists in the Netherlands have recently found that mixed farming, combining arable with dairy farming, leads to less heavy metal contamination than farming based exclusively on one or the other of these. The team calculated contamination levels by examining the difference between the input and output of heavy. They developed indicators for which metal exceeded the quality norms for soil, crops or ground water at any given moment. This makes it possible, for example, to predict that in a country like the Netherlands, given current input and output levels, conventional arable farming will exceed the quality norm for cadmium contamination within 70 years.

3.1 GRAIN SIZE AND ITS EFFECT ON METAL ANALYSIS

In recent years there has been a significant debate within the science community as to the effect of grain size on the adsorption of heavy metals in sediments. Particle dimension is one of the most significant parameters influencing trace metal levels in sediments. Bioavailable sediment –bound metals depend, to a significant extent, on the particle size fraction with which a metal is associated. Traditionally, the fine-grained (silt and clay) fraction of the sediment has been used to examine metal

contamination in the whole sediment sample (Tam & Wong, 2000).

Tam & Wong (2000) designed their study to compare the concentrations of heavy metals bound in the fine-grained fraction (<63um) and the sand-sized fraction (2mm-63um) of the sediments. They found that the highest percentage of sediment in the fine-grained fraction (43%). They suggested that the concentrations of organic matter in the fine-grained fraction of the sediment were often higher than that in the sand-sized fraction. Chakrapani & Subramanian (1993 quoted in Tam & Wong, 2000) reported that Cu, Zn, Mn and Fe increased in concentration with finer size and there was no significant variation in Pb with changes in grain size. The results of Tam & Wong's (2000) study found that the metal concentrations in the swamps represented the natural values and could be considered as the background. Although more metals were retained in the fine-grained sediments in most samples, metals would be accumulated in the sand-sized fraction if the swamp received heavy metals from anthropogenic inputs.

A very considerable amount of work has been reported on the sorption of trace metals by clays. However, increasing evidence suggests that clays are likely to exist only as substrates and will have little or no affect on the uptake of metals.
The metals in this fine-grained fraction are more likely to be biologically available than those in bulk sediments (Bryan & Langston, 1992). Previous workers stated that the clay fraction is more important substrate for metal attachment and metal concentrations tended to increase from sand to silt (up to a 2 fold increase), whereas, the increase from silt to clay averages a 4-5 (Deeply & Fergusson, 1994 quoted in Tam & Wong, 2000).

Physical properties include texture (proportion of sand, silt and clay) and to some extent the type of clay minerals. It is infrequent that predominately coarser-textured soils and sediments become contaminated with problem levels of trace and toxic metals because such minerals have a low affinity for these elements.

However, it has also been argued that concentrations in this selected fraction do not necessarily reflect the concentrations in the whole sediment sample.

Many studies have shown that the highest concentrations of metals are associated with fine grained sediment particles, the trace element being mainly present in the clay – silt particles with grain size less than 63 um, due to the increase in specific surface properties of this fraction. Anomalous fraction distribution of trace metals were found, where the metal concentrations did not increase with decreasing particle size and the highest metal concentrations were in the silt coarse and larger size fraction. These exceptions demonstrate that metal concentrations were not controlled exclusively and mineralogical composition, degree of surface oxidation and organic matter level influencing trace metal content in a sediment fraction.

Haque & Subramanian (1982 quoted in Tam & Wong, 2000) recorded that metal absorption capacity was in the order of sand< silt< clay, due to increases in surface area, minerals and organic matter as particle size decreased from sand to clay. However, this trend of more metal being accumulated in the fine-grained of the sediment may not be universal for all metals and may be varied between metal species

The differences between these two fractions became less significant in sediments which were more contaminated with organic pollution, suggesting that excess organic matter would be accumulated in bulk sediments, not only in fine-grained fraction. In most sediments, the fine-grained fraction contained higher concentrations of heavy metals than the sand-sized fraction. This indicates that more heavy metals were bound in the silt and clay fractions than the sand-sized fraction of the sediment. Except for when the regions pollution load increases dramatically. As pollution increases the metals become less selective about their binding sites and the difference between the two fractions becomes less obvious.

Exceptions exist such as where high concentrations of metals are released directly to a coarse-textured soil resulting in metals contamination of shallow groundwater in coarse-textured subsurface soils (Kotuby-Amacher *et al*. 1992 quoted in Gambrell 1994). Thus, it is the fine-textured soils and sediment containing an appreciable organic matter content that tend to accumulate contaminations in wetlands and surface water bodies.

The manipulation of data usually aims at the elimination of some well known physical and chemical factors which affect the metal distribution process in marine sediments. Since trace metal concentrations usually increase with decreasing grain size of sediments, compensation for grain size differences is one of the most important normalisation techniques for the detection and quantification of anomalous metal concentrations (Angelidis & Aloupi 1997). The correction for grain size differences is based on the tendency of trace metals to be concentrated in fine-grained sediment. Therefore, the coarser material in the sediments plays the role of the "diluent" affecting the total (bulk) metal concentration. There are several approaches to solve this problem; remove the finer sediment fraction through sieving and analyse it separately. This is not exactly a normalisation method and may be used when the fine material is abundant. This is not the case in coastal sediments, where the fine fraction (i.e. silt + clay < 63um) may represent a small percentage of the bulk sediments (Angelidis & Aloupi 1997).

3.2 SAMPLING METHODS

All sediment samples were taken using a small stainless steel Van-Veen Grab. One of the main advantages of using a smaller design is that it is manually controlled. At the surface the jaws are pushed open and kept in that position by a hook which makes the grab very straightforward to use. All the grabs for this study were taken by 1 person while another captured the sample in an appropriate container. By using a smaller apparatus, less time and energy was expended in getting a more than adequately sized sample.

Despite the depth of some of the off-shore sampling sites, sampling was effectively fast and simple using this grab. The grab was equipped with a wire mesh on top so as to reduce or even prevent down drag. This is where the apparatus is slowed down by the wash as it descends to the bottom. When this happens, the sampler is usually slowed down so much that it is unable to take its full bite of the sediment. This requires the operation to be executed all over again.

The samples were then transferred for the jaws of the grab to clean plastic Ziploc© bags. Great attempt was taken not to touch the sample itself as not to taint it in any way. If assistance was required, gloves were worn for protection as well as suppression of contaminants. The volume of the grab was 0.5 litres so smaller bags were adequate and the many samples collected did not take up to much space on the boat. At the end of each sampling day, the samples were transferred to a large freezer for preservation until required for further analysis.

The majority of samples for this study were taken from 12th - 25th May 2005 from the vessel Anibal. However, some other samples were collected from17th - 20th August 2004 from the vessel Urracia and were frozen until required for analysis for this project. The latter samples are all labelled with a letter A-K or a various number. The samples taken this year are also numbered, 1 – 39, but are preceded by the letter K to differentiate between them. The different date of collection will be made apparent later in the study.

The pattern of sampling was devised to take all aspects into account in order to obtain a complete summation the heavy metal levels in the archipelago. Out of the 61 samples, taken a small number of samples (5) were taken on the shores of the inhabited islands where heavy metals levels are thought to be high. 7 samples were taken inside or at the mouth of small estuaries on the islands where rivers flow from elevated positions. These samples were mainly concentrated around San Jose and Isla Del Ray. As stated above, the majority of the samples from 2004 were taken from within and around the islands (K1 - K14 and K16 – K26). The samples labelled T1 – T10 (aka; K15 and K26 – K35) were taken along transect in a north westerly direction from the vicinity of the islands into Punta Mala Bay in Panama Bay. This pattern was devised to

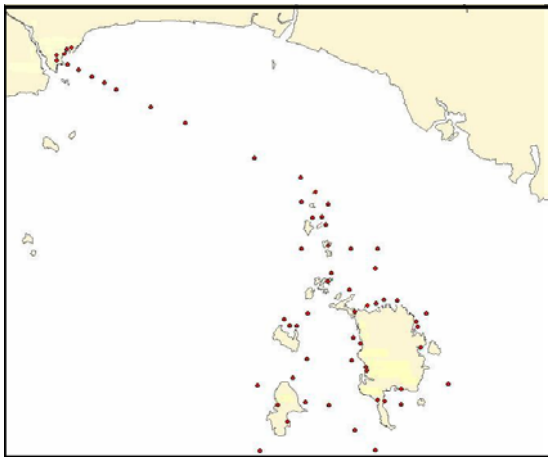


Figure1: Sampling pattern around Las Perlas Islands

give a profile of pollution from the islands outwards and also to investigate if there is a detectable supply of pollution coming directly from the bay out to the islands. From the profile one can also investigate the influence of currents around the Archipelago. One samples (K36) was taken directly from Punta Mala Bay, which was the sampling site for Defew *et al*. (2004) and Benfield, S. (2003), the end of the transect. Three samples (K37 – K39) were taken from inside Panama Bay to investigate the amount of pollution inside the bay and directly available to the Gulf, and subsequently, the Archipelago, by way of tidal currents.

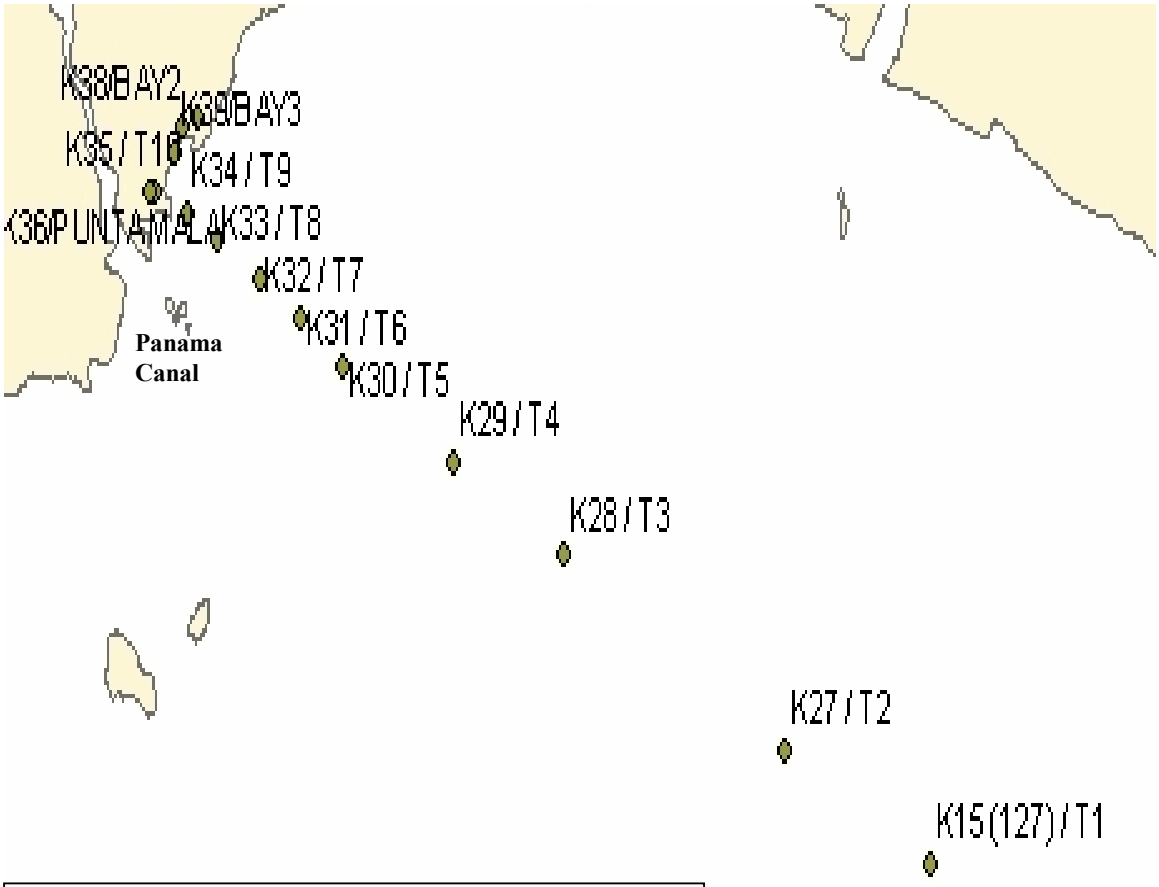


Figure 2: Diagram showing the ten transect stations and four within the bay in which the Canal mouth is clearly marked.

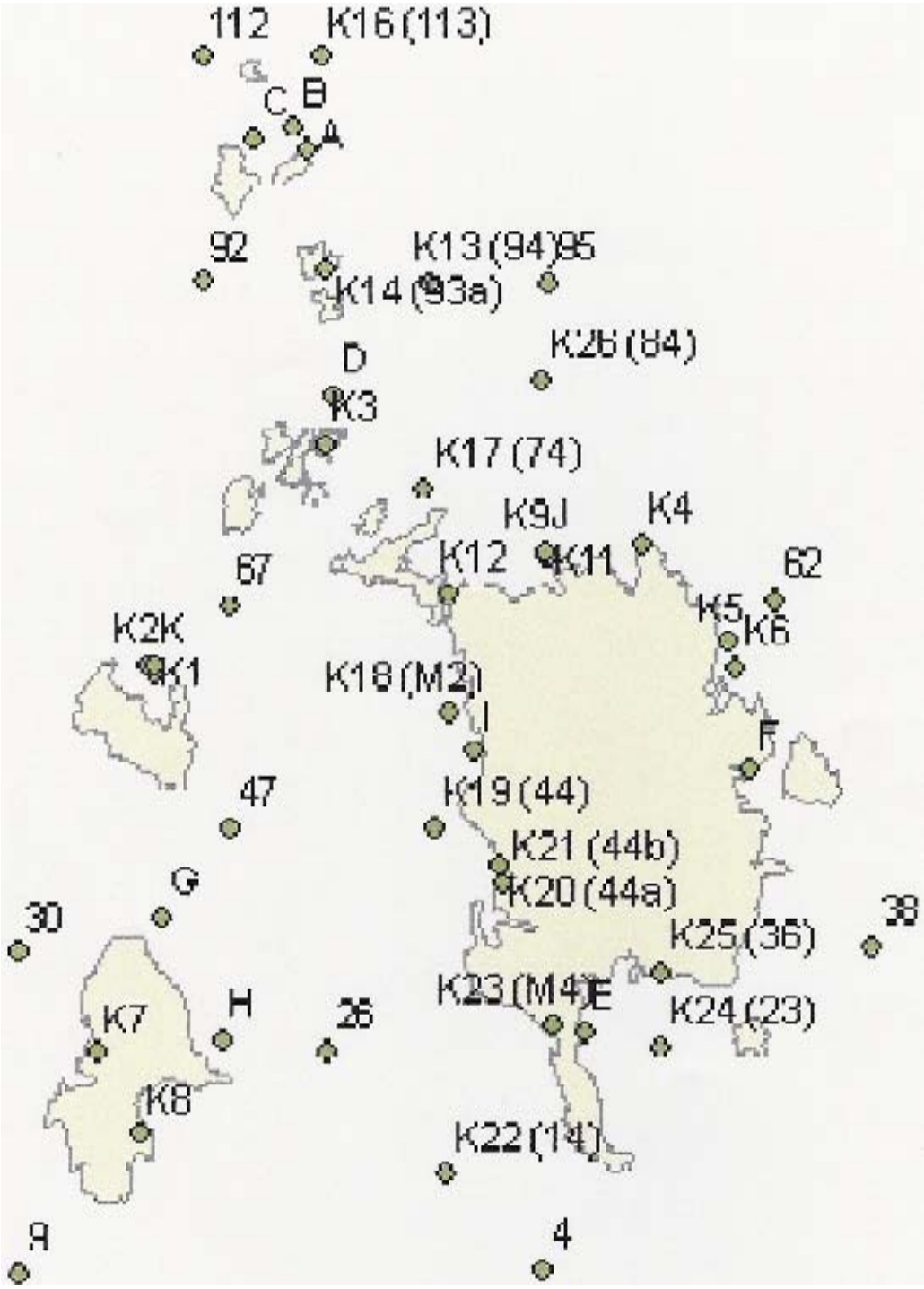


Figure 3: Map showing the metal stations within the Archipelago.

3.3. PARTICLE SIZE ANALYSIS

The previous section in this review has illustrated the importance of grain size on the uptake of heavy metal pollutants. Tropical locations are well known for their fine sanded beaches and due to this these regions are more vulnerable to heavy metal loads.

Part of this study is to ascertain if there is any correlation between the metal levels and grain size of the samples taken. The particle size analysis in both Nov 2004 and June 2005 was completed by a research technician, Heidi Collazos, in Naos Laboratories in Panama City.

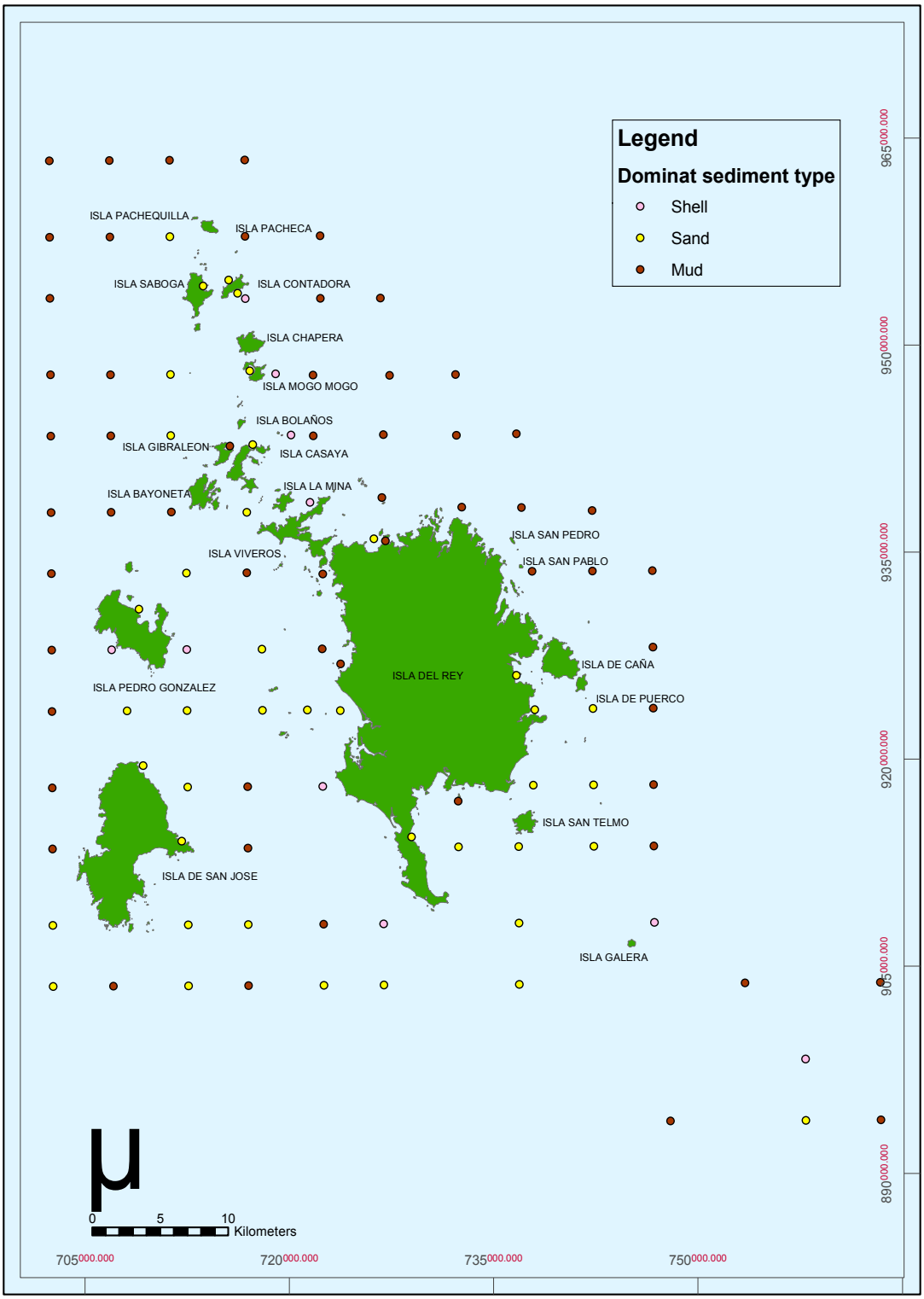
All samples were taken in triplicate for metal analysis and a further replicate for particle size analysis. The wet sieving method was used. After the samples were thawed they were separated into the different fractions using a range of sieves with different mesh sizes. There was some overlapping from stations collected in 2004 and those collected in 2005. Reiteration of information was kept to a minimum during all procedures and reviews. There are 61 samples altogether, 8 of which are in duplicate and 1 in triplicate.

The percentage size of each fraction was calculated using the starting weight of the sample and the separate weights of each fraction. The percentages for organic content, carbonate content, granule, very coarse sand, coarse sand, medium sand, fine sand very fine sand and silt-clay are given for each sample. All the data is available in the Appendix section of this report.

The following diagram shows the results of the GPS mapping of particle distribution in the Archipelago by Sarah Benfield, a Masters student from Heriot Watt, 2003. All results of the present study of particle distribution will be discussed in a later chapter.

Figure 4: Shows a general distribution of sediment types in the Archipelago.

Courtesy of Sarah Benfield.



3.4. METAL ANALYSIS METHODS

The choice of a particular analytical method is most often dictated by the available equipment and facilities. In any trace metal analysis method the first consideration should be the sensitivity of the method. One definition for sensitivity in atomic absorption spectrometry is the concentration of an element that will produce absorption of 1% generally expressed as ug/ml/1%. The detection limits are usually defined as twice the background. The analyst must realize that the stated values for sensitivity and detection limits can be largely instrument and operator dependant and in all cases should be determined experimentally and carefully defined.

The selectivity of analytical methods is the degree to which the method analyses one element with no interference or cross contamination from other elements in the matrix. Ideally, a method that is specific and measures each element individually with little or no interferences would be preferable. Accuracy and precision of the trace metal procedures are important but data will be less accurate as the concentrations analysed reach the ug/l region. Many authors praise the use of inter-laboratory analysis to check standard samples and procedures but Minear & Murray (in the book Signer) do not suggest engaging in that procedure as inter-laboratory cooperation have not always produced outstanding results.

1. After the sediments were fully thawed they were separated using a large sieve to remove any large stones, pebbles and organic matter.

2. They were then placed in acid-washed containers and placed in an oven @ 60°C $\pm$ 5 ^{o}C for 24 hours. Any samples not fully dried after these intervals were given some extra time in the oven until the required amount of drying was achieved.

3. The samples were then fully crushed to the finest possible fraction using an acid-washed pestle and mortar.

4. 2g of this crushed sediment was then transferred to a sample, acid-washed

beaker into which 10ml of 70% Reagent grade nitric acid was added and the beaker covered with a watch glass. The amount of effervescence observed indicated the amount of organic material within the sample. The samples were left overnight to digest completely at room temperature.

5. 20ml of distilled water was added to each beaker when they were adequately cooled and placed into a temperature controlled water bath @ $60^{o}C \pm 5\ ^{o}C$ for three hours. Water bath is more desirable in this situation instead of a hot plate as it regulates the temperature better and distributed the temperature evenly.

6. After the samples had all cooled to room temperature; they were all filtered through a glass funnel containing Whatttman No.1 filter paper. The expected concentration of the sample dictated the size of the volumetric flasks used. The reaction vessels and watch glasses were rinsed with distilled water to recover any residual metals. 100ml, 50ml and 25ml volumetrics were all used and made up to the mark with distilled water if need.

7. Analysis was performed by Atomic Absorption Spectroscopy with the use of standards to allow determination of metal concentrations within each sample.

(Method taken from Defew *et al*.,2004 and White.E, 2004)

Atomic Absorption Spectrometry

AAS is an important technique for analyzing metals in water. The general method involves atomization of samples by thermal sources and the absorption of a specific wavelength by the atomic source as it is excited. The radiation used is a hollow cathode lamp containing, as its cathode, the same element under analysis. The quantity of the same element absorbed by the atomic vapor is proportional to the concentration of the atoms in the ground state.

4.1. RESULTS OF PARTICLE SIZE ANALYSIS

K1	V C Sand	T2 (K27)	Silt - Clay	9	V F Sand
K2	Granule	T3 (K28)	Silt - Clay	26	F Sand
K3	Silt - Clay	T4 (K29)	Silt - Clay	30	V F Sand
K4	F Sand	T5 (K30)	Silt - Clay	38	M Sand
K5	F Sand	T6 (K31)	Silt - Clay	47	F Sand
K6	F Sand	T7 (K32)	Silt - Clay	62	Silt - Clay
K7	V F Sand	T8 (K33)	Silt - Clay	67	C Sand
K8	F Sand	T9 (K34)	Silt - Clay	92	V F Sand
K9	Granule	T10 (K35)	F Sand	95	Silt - Clay
K10	Granule	K36 (Pta. Mala)	F Sand	112	Silt - Clay
K11	Granule	K37 (Bay 1)	Silt - Clay		
K12	Silt - Clay	K38 (Bay 2)	Silt - Clay		
K13 (94)	Silt - Clay	K39 (Bay 3)	Silt - Clay		
K14 (93a)	C Sand	A	F Sand		
K16 (113)	Silt - Clay	B	M Sand		
K17 (74)	V C Sand	C	C Sand		
K18 (M2)	Silt - Clay	D	C Sand		
K19 (44)	F Sand	E	V F Sand		
K20 (44a)	M Sand	F	V F Sand		
K21 (44b)	M Sand	G	M Sand		
K22 (14)	M Sand	H	M Sand		
K23 (M4)	M Sand	I	Silt - Clay		
K24 (23)	F Sand	J	Silt - Clay		
K25 (36)	V F Sand	K	V C Sand		
K 26 (84)	Silt - Clay	4	M Sand		
T1 (K15, 127)	Silt - Clay	9	V F Sand		

4.2. METAL ANALYSIS

All the results of the Atomic Absorption Spectrometry are shown belowin table . Only eight metals were analysed as opposed to the ten that were proposed earlier. The metals vanadium (V) and mercury (Hg) were rejected for two reasons.

1. Nitrous oxide gas is required for analysis of vanadium whereas all the other metals are analysed using oxygen. As this gas was unavailable at the time of analysis, it was discarded.

2. A mercury cathode lamp was also unavailable at the time of analysis and was, for this reason, also discarded.

The highest and lowest concentration of each metal is highlighted for easy locating.

Sample	Cu	Cr	Cd	Fe	Zn	Ni	Pb	Mn
K1	28.62	10.25	5.25	56107.5	72.5	17.37	8.125	21.5
K2	21.25	10.62	5.78	17788.12	46.93	15.62	46.62	26.5
K3	14.87	15	3.62	102416	54.04	21.47	9.12	31.75
K4	14.25	20	3	240.09	54.1	12.87	8.25	19.75
K5	1.75	14.125	2.87	191.71	46.29	5	32	9.25
K6	9.37	13.37	1	207.92	38.29	10.87	33.25	0.25
K7	6.625	19.62	1.5	237.33	56.72	15.37	30.87	0.25
K8	8.75	25.25	5.25	182.95	23.25	19	49.5	16.5
K9	45	22.5	3.25	89.3	19.8	13.5	102.5	17.75
K10	57	27.5	2.25	376.1	129.99	16.25	164.75	18.5
K11	32.5	25.75	2.5	386	224.84	15.25	125.25	3.5
K12	32	28.5	2.5	400.37	51.05	16	58.5	53.25
K13 / 94	13.5	33	1.87	243.25	56.26	18.62	63.87	121.25
K14 / 93A	11.12	13.87	3.37	136.12	38.23	12.25	83.87	17.5
K15 / T1 / 127	16.75	24.75	2.5	221.51	51.69	16.25	120.37	45.25
K16 / 113	11.12	18.5	3.75	178.36	44.67	13.25	151.62	25.5
K17 / 74	5.25	22.5	6.5	115.92	18.51	15.5	358.5	19
K18 / M2	8.62	22	4.5	457.55	44.68	11.25	150.87	121.25
K19 / 44	9.5	17	7.25	392.15	36.87	12.25	124.87	132
K20 / 44A	10.75	26.25	7.75	218.07	16.3	15.5	310.25	72.75
K21 / 44B	7.75	18.75	5.5	65.3	13.9	13.75	286	76.5
K22 / 14	33.5	35.5	5	408.92	51.02	23.25	306	794.5
K23 / M4	10	21.5	8	11.35	13.24	21.25	234.5	83.5
K24 / 23	14.5	21.25	6.5	256.9	23.94	16.75	277.25	0.75

K25 / 36	13.95	19.5	7.75	66.75	25.97	19	183.5	12.5
K26 / 84	36.75	60.5	5.5	474.6	87.42	36	286.25	1.5
K36 / Punta Mala	15.5	20.75	4.75	429.05	69.845	14.75	254.25	1.75
K37 / Bay 1	66	23.5	6	392.92	119.65	18	372.75	195
K38 / Bay 2	40.75	21.75	6	380.3	93.82	10.75	335.25	210
K39 / Bay 3	94	38.25	6	428.87	133.4	20.75	378.75	270
T1 / 127 / K15	16.75	24.75	2.5	221.51	51.69	16.25	120.37	45.25
T2 / K27	23.62	30.375	1.62	438.02	58.12	12.75	173.25	36.5
T3 / K28	28.12	40.12	1.62	231.11	48.96	15.5	149.75	12.875
T4 / K29	20.12	32.12	1.5	227.74	54.84	16.12	122.87	13.375
T5 / K30	23.37	24.75	1.25	235	53.75	14.12	172.75	14.375
T6 / K31	22.62	24.87	1.62	237.63	56.95	13	142.12	165.75
T7 / K32	22.87	19.75	1.75	232.01	54.19	11.5	135.87	235
T8 / K33	25.12	16.12	2	220.65	59.88	11	177	186.87
T9 / K34	17	7	2.75	238.11	59.02	11.87	134	298.62
T10 / K35	11.62	1.62	2.25	216.43	53.24	12.37	121.5	337.5
A	7.5	24.37	3.5	120.13	22.42	6.62	126.5	40.75
B	8.37	24.25	3.87	143.78	29.22	13.12	81.87	45.37
C	7.25	26.87	3.87	80.8	27.15	12.25	111.37	15
D	11.75	26	4.12	103.32	27.2	112.5	97.75	15.75
E	19.12	17.25	1.5	23.8	29.22	11.87	109.75	134.87
F	13.87	24.62	1	204.89	39.76	8.37	124	86.25
G	7.87	24.5	3.5	128.13	25.29	9.12	77.37	33.12
H	7.12	17.37	3.5	88.38	22.93	7	89.75	24
I	12.62	14.87	1.25	227.76	25.6	7.62	98.25	155.5
J	28.5	21.25	0.75	242.03	50.86	9.75	112.75	138.25
K	8.62	5.5	2.62	33.66	9	9	77.62	29.37

4	11.87	13.62	2	200.02	14.68	13.75	79.5	180.87
9	7.35	14.37	1.5	182.64	17.93	10.37	77.37	112.25
26	9.37	8.5	1.12	198.58	19.51	7.87	96	120.62
30	16.75	16.75	2.25	222.47	29.74	14.25	98.62	77.62
38	9.125	6.12	3.12	139.59	6.46	6.87	73.37	63.75
47	9.12	14.62	1.12	240.72	20.41	6.87	100	61.12
62	22.62	14.75	0.87	224.46	24.29	12.37	127.25	106.62
67	11.25	22.62	1.5	255.72	21.65	7.87	111.37	111.5
92	9	9.12	3.37	165.26	8.04	9.5	98.87	39.37
95	28.25	28.5	0.75	230.25	21.09	11.62	129.12	85.58
112	19.5	10.25	2.25	169.38	5.6	18.12	90.75	40.5

Table1: All results (mg / kg) obtained from AAS for each metal at each station

Blue → denotes the highest concentration for the analysed metal

Yellow → denotes the lowest concentration for the analysed metal

The atomic absorption spectrometer does not show the results in the required concentrations (mg / kg) so a number of equations were used to modify the given results.

Due to a small discrepancy in the machine, the results for Zn were given in the form of Absorption as opposed to Concentration like all the other metals. Becanse of this a slightly different equation was used for Zn.

Zn →

$$\text{Standard Concentration (ppm)} \times \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times \frac{\text{Sample Volume (ml)}}{\text{Dry Weight Extracted (g)}} \times \text{Dilution}$$

Cu, Cd, Cr, Fe, Mn, Ni, Pb →

$$\frac{\text{Concentration (ppm)} \times \text{Dilution} \times \text{Sample Volume (ml)}}{\text{Dry weight Extracted (g)}} = \text{mg / kg}$$

All equations and full results are shown in the Appendix section along with all the calibration curves used to calibrate the AAS for metal analysis. Equations obtained thought personal consultation with Sean McMenamy and Dr. Bev Barras.

For the table above it can be seen that the results do not show the expected patterns of contamination that were expected. The concentrations for **Cadmium** in the sediments of Las Perlas ranged from 0.75 to 8 mg kg^{-1}. The concentration range for **Chromium** was seen to be quiet similar, 1.62 to 60.5 mg kg^{-1}. **Nickel** and **Copper** also showed respectable concentrations in the sediments, 5 to 23.25 mg kg^{-1} 1.75 to 94 mg kg^{-1} respectively.

The other four metals demonstrated more unusual results. **Manganese** concentrations ranged from 0.25 to 270 mg kg^{-1}. **Lead** concentrations, 8.125 to 378.75 mg kg^{-1} and **Zinc** concentration are 5.6 to 224.84 mg kg^{-1}. **Iron** concentrations are 23.8 to 17788.12 mg kg^{-1}.

5.1. INTERPRETATION OF RESULTS

5.1.1 MEAN CONCENTRATIONS

The mean concentrations for each metal, over the entire span of the sampling sites are shown in the table below.

All		**Islands**		**Transect**		**Bay**	
Cd	**3.31**	**Cd**	**3.41**	**Cd**	**1.89**	**Cd**	**5.69**
Ni	**15.3**	**Ni**	**15.63**	**Ni**	**13.45**	**Ni**	**16.06**
Cu	**19.18**	**Cu**	**15.87**	**Cu**	**21.12**	**Cr**	**26.06**
Cr	**20.95**	**Cr**	**20.28**	**Cr**	**22.15**	**Cu**	**54.06**
Zn	**45.26**	**Zn**	**38.30**	**Zn**	**55.06**	**Zn**	**104.18**
Mn	**88.44**	**Mn**	**72.09**	**Mn**	**134.61**	**Mn**	**169.19**
Pb	**138.49**	**Pb**	**120.74**	**Pb**	**144.95**	**Pb**	**335.25**
Fe	**3058.47**	**Fe**	**3864.50**	**Fe**	**249.82**	**Fe**	**407.79**

Table 2: Shows the means concentration of each metal analysed (mg kg^{-1}).

As can be seen from the table above, metal levels in all areas of the sample sites exist in the order Cd >Ni>Cu>Cr>Zn>Mn>Pb>Fe. The only exception is within Panama Bay where Cr is seen to have a larger concentration than Cu.

5.1.1.1 Cadmium (Cd)

Cadmium has been found to be the metal with the lowest concentration found in the marine sediments of Las Perlas. Four standards were used to calibrate the apparatus before analysis all of which were lower than 2ppm. All the samples were within this range so no further dilutions were required. As can be seen from the calibration curve (Appendix 2) the correlation coefficient was 0.999 which illustrates that all results were within 0.001% of their true value. The levels of cadmium found are low, 3.31 mg kg^{-1} overall, 3.41 mg kg^{-1} around the islands, 1.89 mg kg^{-1} along the transect and at its highest concentration, 5.69 mg kg^{-1} in Panama Bay. All concentrations except that of the Bay are low and can be attributed to natural background levels. Therefore it can be deduced that cadmium poses little or no treat to this most of this environment.

5.1.1.2. Nickel (Ni)

Like Cadmium above, four standards were used to calibrate the spectrometer for nickel. These were 2ppm and lower and all samples analysed were within this range so no further dilutions were necessary. The correlation coefficient was 0.993 and illustrates that samples were within 0.007% of their true value. According to the Swedish EPA, marine sediment contaminations are indicated by a value higher than 100 mg kg^{-1}. The concentration found are quiet low, 15.3 mg kg^{-1} overall, 15.63 mg kg^{-1} in and around the islands, 13.45 mg kg^{-1} along the transect and 16.06 mg kg^{-1} with the Bay. Using these guidelines, the nickel levels found in Las Perlas are well beneath contamination levels, can be attributed to natural background levels and pose no threat to the surrounding environment.

5.1.1.3. Copper (Cu)

Five standards, 5ppm and below, were used to calibrate the spectrometer before copper analysis commenced. The majority of samples were within the range and only a few dilutions were required. These dilution factors were taken into account in the

appropriate equations when results were analysed. The levels of copper found in the sediments of Las Perlas, 19.18 mg kg^{-1}overall, 15.87 mg kg^{-1}, 21.12 mg kg^{-1} along the transect and 54.06 mg kg^{-1} in the Bay are well below the contamination levels stated by the Swedish EPA, 80 mg kg^{-1}. Therefore all levels can be considered background and pose n threat to the local environment.

5.1.1.4. Chromium (Cr)

In this instance three standards were used to calibrate the spectrometer before chromium analysis. All the standards used were 3ppm and under and as all samples were within this range no further dilutions were needed. As the calibration curve shows, the correlation coefficient was 0.997 which indicates that all samples were within 0.003% of their true value. The Swedish EPA claims that the level of chromium contamination is 70 mg kg^{-1}. Since the concentrations found in Las Perlas are below this, 20.95 mg kg^{-1} overall, 20.28 mg kg^{-1} in and around the islands, 22.15 mg kg^{-1} along the transect and 26.06 mg kg^{-1} in the Bay. All the concentrations can be attributed to natural background levels. Therefore, chromium poses no threat to the surrounding environment.

5.1.1.5. Zinc

Six standards were used to calibrate the spectrometer before zinc analysis, the highest of which was 2ppm used with lower concentrations. All samples were within this range so no further dilutions were required. The Swedish EPA states that zinc concentration must be above 360 mg kg^{-1} to be considered contaminants. Levels in Las Perlas are found to be 45.26 mg kg^{-1} overall, 38.30 mg kg^{-1} in and around the islands, 55.06 mg kg^{-1} along the transect and 104.18 mg kg^{-1} in the Bay. All concentrations can be considered to be natural background levels and present little or no environmental effects.

5.1.1.6. Manganese (Mn)

Four standards, 2ppm and below, were used to calibrate the spectrometer before

analysis of manganese. The majority of samples were within this range and any further dilution factors were taken into account. There are currently no contamination levels available for manganese with Europe or the Americas. The concentrations found in Las Perlas are 88.44 mg kg^{-1} overall, 72.09 mg kg^{-1} in and around the islands, 134.61 mg kg^{-1} along the transect and 169.19 mg kg^{-1} in the Bay. However judging by other articles and studies available, it would appear that these levels pose no threat to the surrounding environment should be closely monitored to prevent any increase. The concentrations with the bay are higher than the rest and would suggest more discharge than the other sampling sites.

5.1.1.7. Lead (Pb)

Given that lead is such a common metal found in the natural environment is was necessary that high concentration standard were used, when calibrating the spectrometer, to ensure that all samples were within range. For this reason four standards were used ranging from 20ppm concentration to 1ppm. The correlation coefficient was 0.979 and the calibration curve can be seen in Appendix 2. All samples were within this range and no further dilutions were required. This is the first of the metals that can be considered a contaminant as levels are above that stated by the Swedish EPA. Levels over 110 mg kg^{-1} are deemed to be higher than background levels and can begin to have a small but detrimental effect on the surrounding environment. Since the levels from Las Perlas are above the standard, they are not extreme, 138.49 mg kg^{-1} overall, 120.74 mg kg^{-1} in and around the islands and 144.95 along the transect although the levels found in the Bay, 335.25 mg kg^{-1} ca be considered as reaching pollution levels. The problem does not appear to be extensive but is still substantial and needs to be monitored further to prevent any permanent damage. It is possible that these levels are elevated by the amount of waste oil in the sediments around the canal mouth and the islands and especially from the Bay and the pollution discharged into it. It is unsurprising then that the highest levels throughout all the sampling sites were found at site K39. This site is in the centre of the Bay. The lowest concentrations were found at site K1 which was taken from the shore at Pedro Gonzalesz. Since this shore is used to harbour local fishing boats and would be open to much wasted oil, it is unexpected that the levels of lead found here are not

higher than those established.

5.1.1.8. Iron (Fe)

Iron was the most difficult of all the metals to analyses. As this metal is the tenth most common element on the plant it is found almost everywhere and as a component of almost all man made products. In conjunction with its popularity iron can also have quite high natural background levels. Three standards were used ranging from 0ppm to 10ppm. The majority of the samples were within this range but the results from a small handful (K1 – K3) of samples were well outside these limits. These samples were diluted as necessary and all results can be seen in Appendix 2 and 3. The mean levels from these sediments was seen to be 3058.47 mg kg^{-1} overall, 3864.50 mg kg^{-1} in and around the islands, 249.82 mg kg^{-1} along the transect and 407.79 mg kg^{-1} in the Bay. As opposed to the above concentrations, the highest levels of iron was found on the shore at Pedro Gonzalez, at station K2. This is not surprising that these levels are found here because of all the activities that take place on this shore. The lowest levels were found at station E, one of the most southerly stations, found on the west side of a small peninsula from Isla De Ray.

5.1.2. CONCENTRATIONS ALONG THE TRANSECT TRANSECT

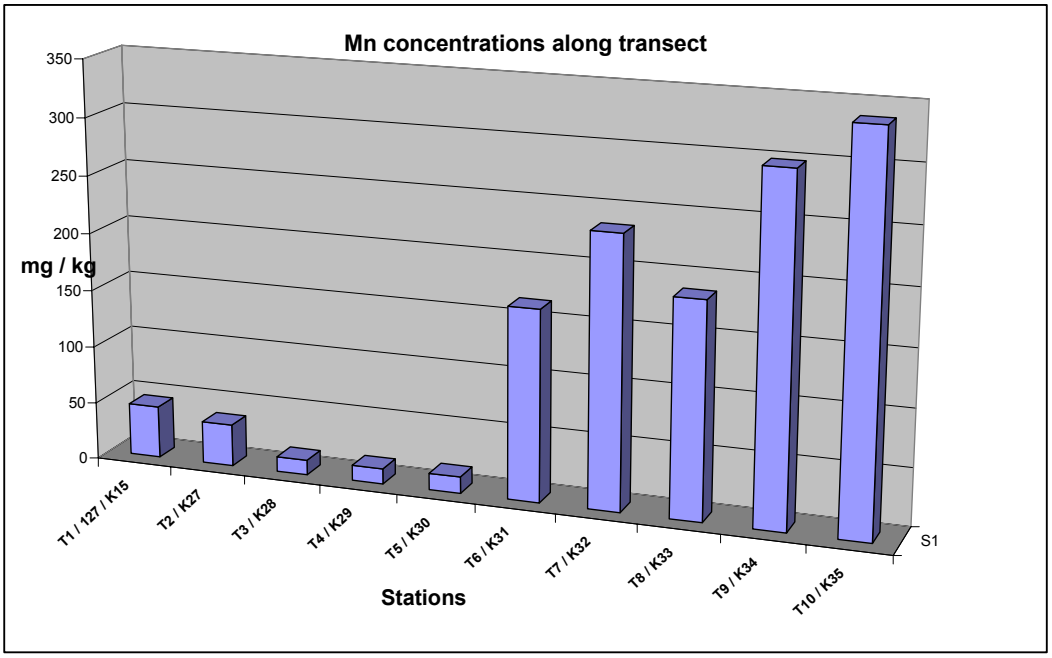
T1 / 127/ K15	16.75	24.75	2.5	221.51	51.69	16.25	120.37	45.25
T2 / K27	23.62	30.375	1.62	438.02	58.12	12.75	173.25	36.5
T3 / K28	28.12	40.12	1.62	231.11	48.96	15.5	149.75	12.875
T4 / K29	20.12	32.12	1.5	227.74	54.84	16.12	122.87	13.375
T5 / K30	23.37	24.75	1.25	235	53.75	14.12	172.75	14.375
T6 / K31	22.62	24.87	1.62	237.63	56.95	13	142.12	165.75
T7 / K32	22.87	19.75	1.75	232.01	54.19	11.5	135.87	235
T8 / K33	25.12	16.12	2	220.65	59.88	11	177	186.87
T9 / K34	17	7	2.75	238.11	59.02	11.87	134	298.62
T10 / K35	11.62	1.62	2.25	216.43	53.24	12.37	121.5	337.5

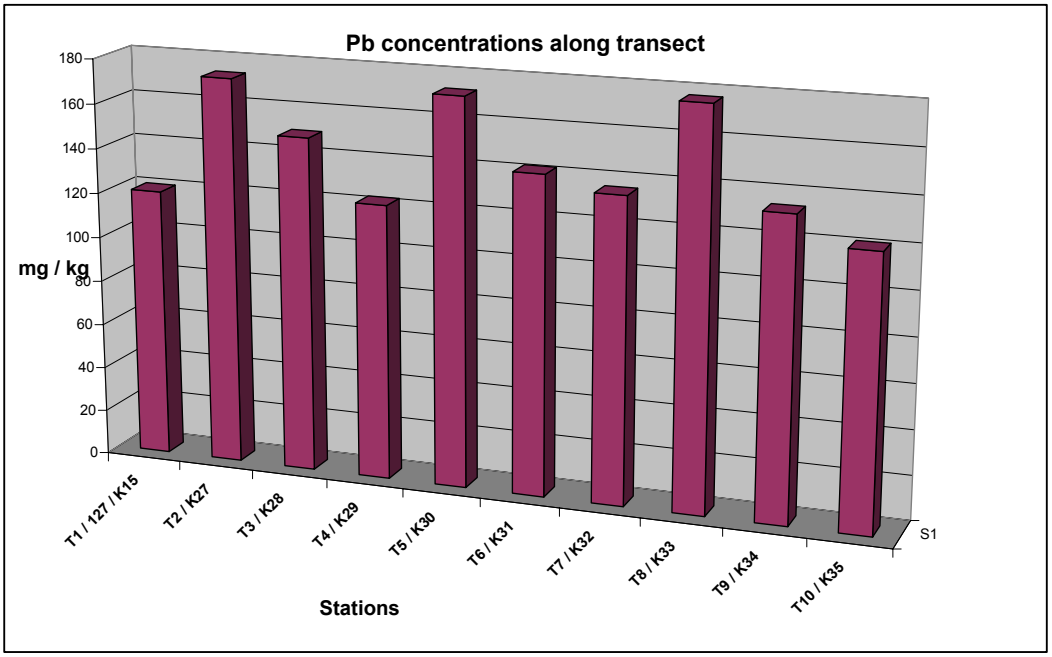
Table 3: All the concentrations for each metal along the transect

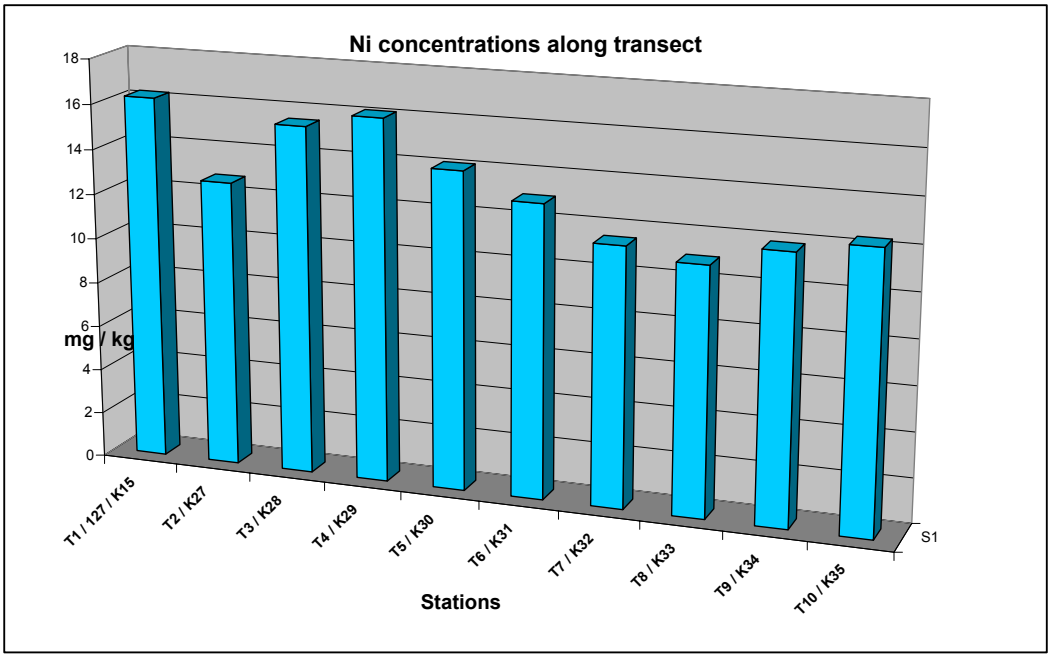
The following graphs depict the concentration of each metal along the transect from the most northerly point of the archipelago to the centre of Punta Mala within Panama Bay. It was expected that the graphs would show the lowest levels of each metal at T1 and gradually increasing until the last station T10. It is evident from the eight illustrations below that this is not the case. The only metal that shows a pattern close to the expected is Mn. Cr shows almost the opposite pattern whereas the other metals exhibit various concentrations and patterns.

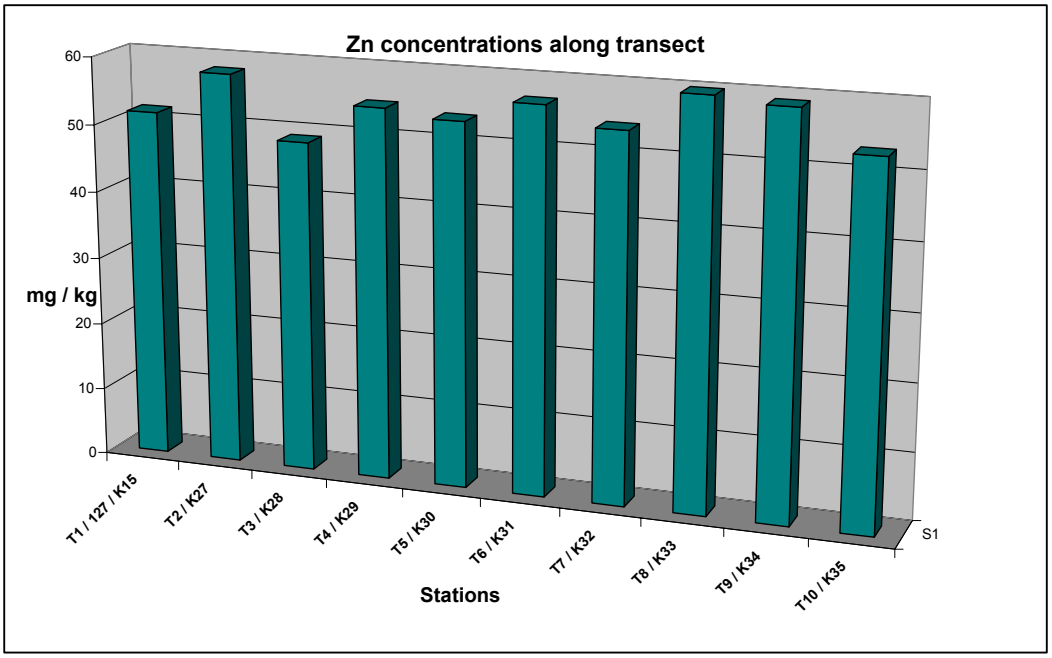
Pb, Ni, Zn, Fe and Cu concentrations remain relatively consistent throughout all of the stations along the transect with a few random peaks or troughs.

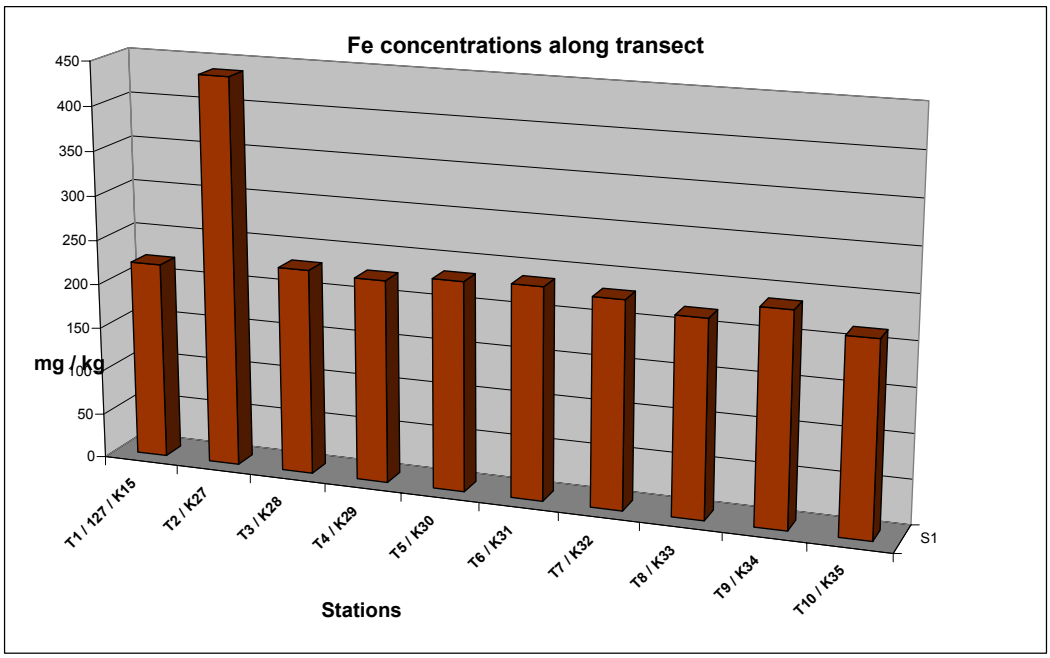
Therefore, it can be determined from these graphs that no constant pattern exists along the transect within this area.

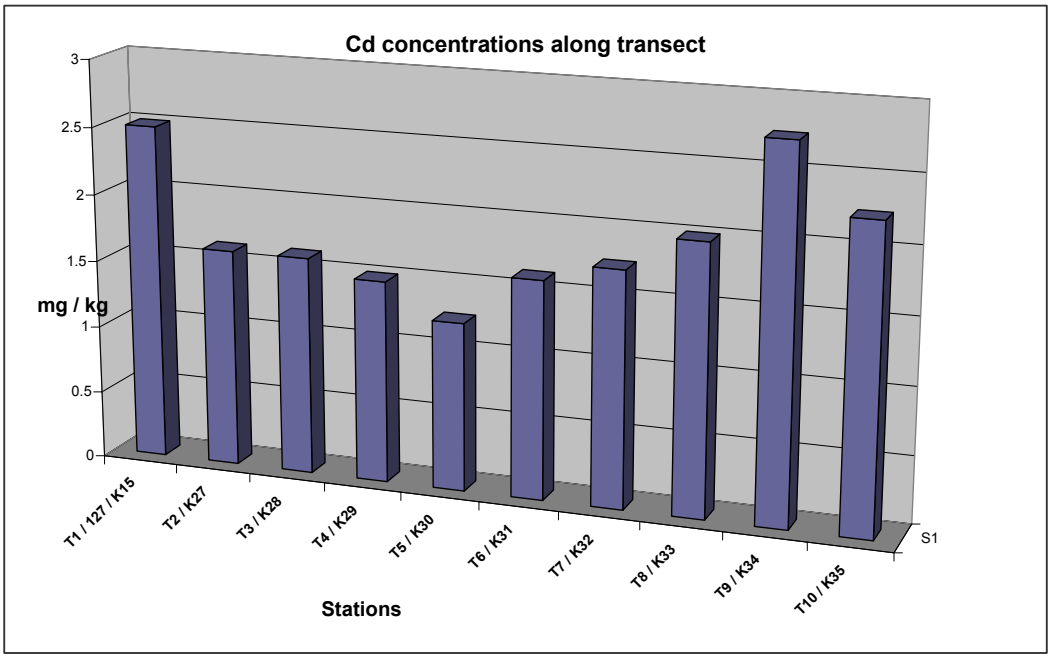

Mn concentrations along transect
mg / kg
350
300
250
200
150
100
50
0
T1 / 127 / K15
T2 / K27
T3 / K28
T4 / K29
T5 / K30
T6 / K31
T7 / K32
T8 / K33
T9 / K34
T10 / K35
S1
Stations

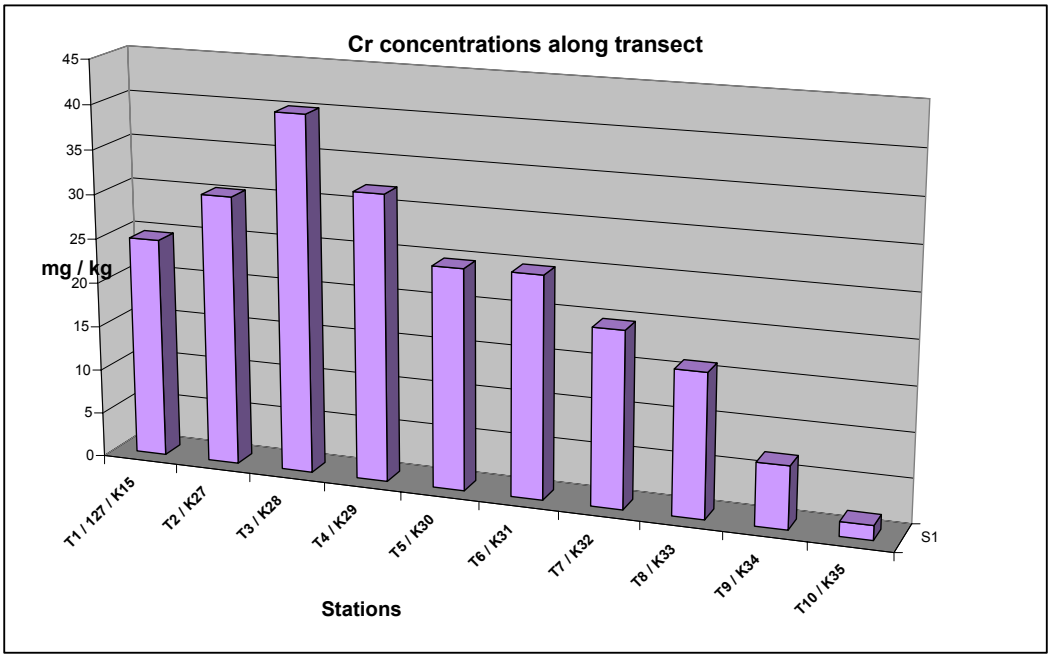

Pb concentrations along transect
mg / kg
180
160
140
120
100
80
60
40
20
0
T1 / 127 / K15
T2 / K27
T3 / K28
T4 / K29
T5 / K30
T6 / K31
T7 / K32
T8 / K33
T9 / K34
T10 / K35
S1
Stations

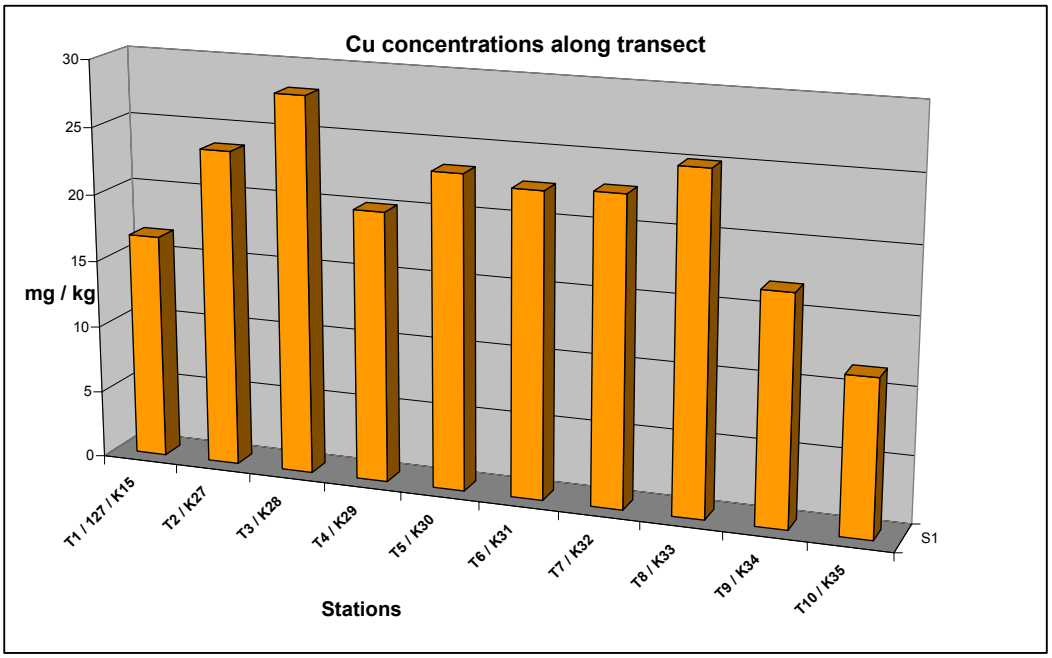

Ni concentrations along transect
mg / kg
18
16
14
12
10
8
6
4
2
0
T1 / 127 / K15
T2 / K27
T3 / K28
T4 / K29
T5 / K30
T6 / K31
T7 / K32
T8 / K33
T9 / K34
T10 / K35
S1
Stations


Zn concentrations along transect
mg / kg
60
50
40
30
20
10
0
T1 / 127 / K15
T2 / K27
T3 / K28
T4 / K29
T5 / K30
T6 / K31
T7 / K32
T8 / K33
T9 / K34
T10 / K35
S1
Stations


Fe concentrations along transect
450
400
350
300
250
200
150
100
50
0
mg / kg
T1 / 127 / K15
T2 / K27
T3 / K28
T4 / K29
T5 / K30
T6 / K31
T7 / K32
T8 / K33
T9 / K34
T10 / K35
S1
Stations

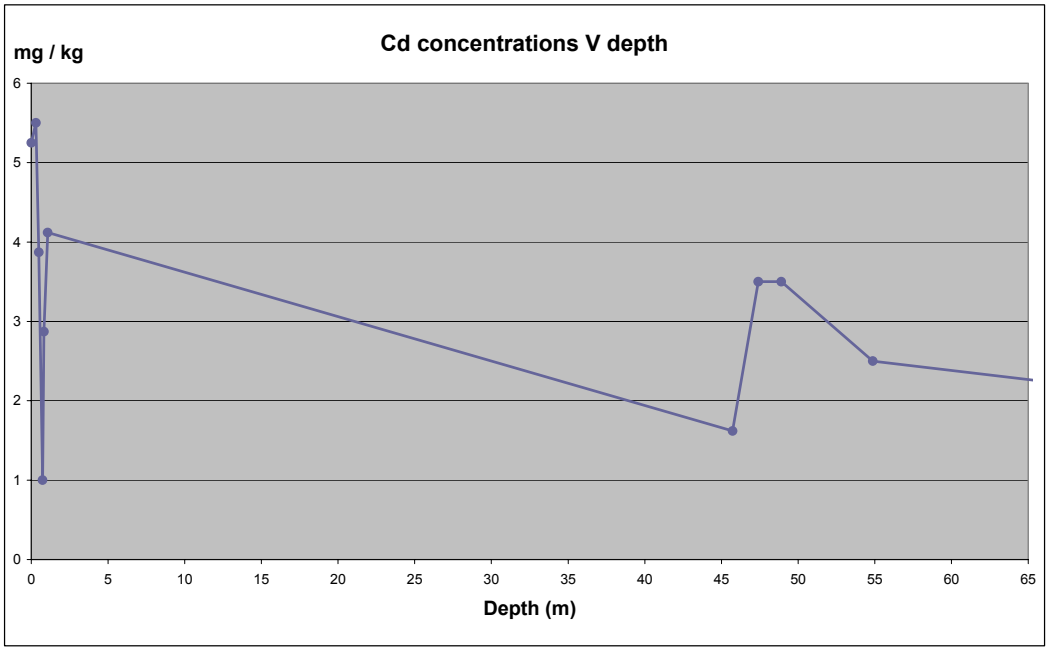

Cd concentrations along transect
3
2.5
2
1.5
1
0.5
0
mg / kg
T1 / 127 / K15
T2 / K27
T3 / K28
T4 / K29
T5 / K30
T6 / K31
T7 / K32
T8 / K33
T9 / K34
T10 / K35
S1
Stations

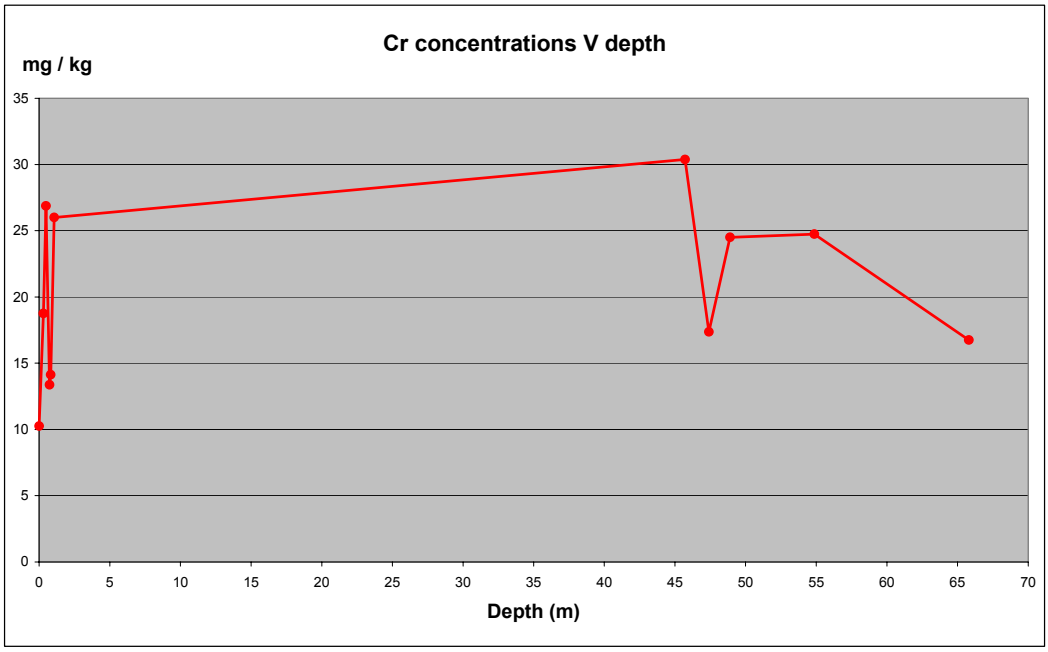

Cr concentrations along transect
mg / kg
45
40
35
30
25
20
15
10
5
0
T1 / 127 / K15
T2 / K27
T3 / K28
T4 / K29
T5 / K30
T6 / K31
T7 / K32
T8 / K33
T9 / K34
T10 / K35
S1
Stations


Cu concentrations along transect
mg / kg
30
25
20
15
10
5
0
T1 / 127 / K15
T2 / K27
T3 / K28
T4 / K29
T5 / K30
T6 / K31
T7 / K32
T8 / K33
T9 / K34
T10 / K35
S1
Stations

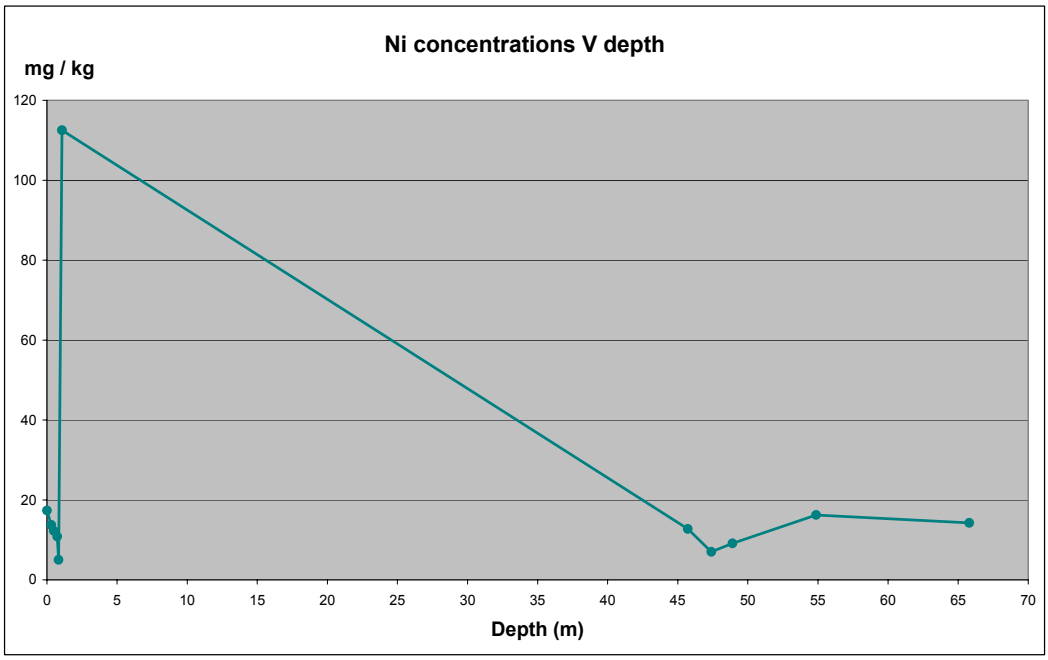
5.1.3. CONCENTRATION AND DEPTH

Three metals, Cd, Cr and Ni were chosen to investigate whether depth had any influence on concentration within the sample. The following graphs show that these metals are concentrated within the first few meters of sediment. It can be seen that cadmium and chromium start with a sharp incline in the first meter as opposed to nickel which begins with a sharp decline. All there of the metals illustrate no correlation between concentration and depth. This may be simply due to the action of settling or current patterns but it can be said that the most concentrated sediments are found closer to the shore. It is not true that shallow sediments have higher concentrations either it simply depends upon discharge points and their proximity the sample sites.





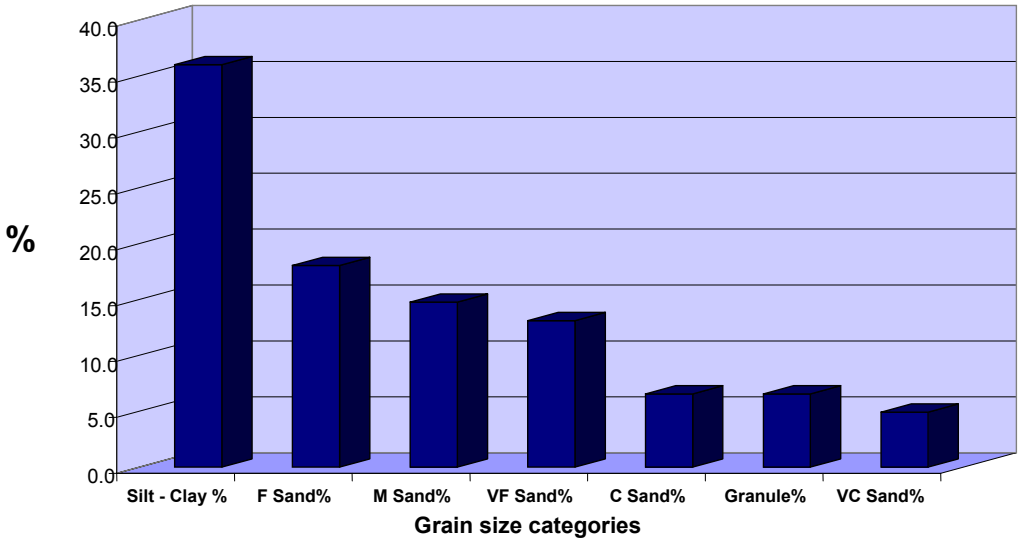
An assessment of heavy metal contamination in the marine sediments of Las Perlas Archipelago, Gulf of Panama



5.1.4. `PARTICLE SIZE ANALYSIS

The worksheet showing the collection of results is available in the appendix chapter of this study.

Figure 5: Grain size distribution (%)



As was expected the most widespread grain size in the sediments of Las Perlas was the silt – clay fraction. This was followed by fine sand, medium sand, very fine sand, coarse sand, granule and finally very coarse sand. The highest percentage, silt – clay is more than seven times higher than the lowest, very coarse sand. The percentage for coarse sand and granule are completely equal, both being 6.6% or 4 samples out of the total 61 samples.

Silt - Clay %	**Fine Sand%**	**Medium Sand%**	**Very Fine Sand%**	**Coarse Sand%**	**Granule%**	**Very Coarse Sand%**
36.0%	**18.0%**	**14.8%**	**13.1%**	**6.6%**	**6.6%**	**4.9%**
36%	**54%**	**68.80%**	**81.90%**	**88.50%**	**95.20%**	

						100%
63 µm	**125 µm**	**250 µm**	**500 µm**	**750 µm**	**1mm**	**2mm**

Table 4: Grain size distribution and class sizes.

The silt – clay fraction consists of particles less than 63µm in diameter. Although this fraction is the highest component of all the samples, it is only accounts for 36% of all the sediment collected, just a little over 1/3. When the results for silt – clay and fine sand are added, it is apparent that over half (54%) of the sediment collected is under 125um. As the categories progress and the particle size increases it is evident that 70% is under 250 µm, 82% is under 500 µm, 88.5% is under 750 µm, 95.1% is less than 1mm and 100% is less than 2mm. Figure 5 and Table 1
above show the percentage distribution of sediments from the Archipelago divided into the seven different categories along with their parameters.

These results concur with the results obtained by Sarah Benfield for Particle Distribution in the Archipelago (which can be found in Section 3.3.). Only three categories were used in that study mud, sand and shell and seven were used in this study. However it has been established (through personal consultation) that mud was considered to be <63 µm. Therefore, the results of both studies are identical

5.2. CONCLUSIONS

This study has satisfied the aims and objectives by analysing the metals concentration of marine sediments taken from Las Perlas and surrounding areas.

The results and concentrations have been as expected but patterns of those concentrations have been surprising and unanticipated. From the results it can be concluded that Las Perlas is a relatively pristine and clean area. The majority of metals show no possibility of detrimental effects on the local environment. These metals, however, must be monitored to ensure that they stay at harmless levels. The

other metals, namely, manganese, lead and iron are very to close to if not above safe levels. The concentrations exhibited may not presently result in any deleterious or harmful effects however; further discharge of said metals, from any source, in any of the areas examined may have dangerous effects on the surrounding environment.

5.3. FUTURE RESEARCH

1. As can be seen from the results and concentrations gathered the majority of contamination is present in the Bay of Panama. This area should be examined further and any pollution sources should be identified.

2. Another transect could also be taken beginning at the islands but ending at the mouth of the canal to examine if pollution is travelling along that direction.

3. It has been suggested by many authors that pH , salinity, temperature and redox potential all affect uptake and retention of metals in the sediments. This aspect should be investigated further to fully comprehend metals in this environment.

6.1. REFERENCES CITED

Angelidis, M.O., Aloupi, M. (1997) Assessment of metal concentrations in shallow coastal sediments around Mytilene, Greece. *Interm. J. Environ. Anal. Chem*. Vol **68** (2), pp 281-293.

Belize, N., Chen, Y., Gunn, J., Dixit, S. (2004) Sediment trace metal profiles in lakes of Killarney Park, Canada: from regional to continental influence. *Environmental Pollution* Vol **130**, pp 239-248.

Benjamin, M.M., Leckie, J.O. (1980) Adsorption of metals at oxide interfaces: effects of the concentration of adsorbate and complexing metals. In: Contaminants and sediments, Vol **2**. (Ed) Baker, R.A. Ann A rbour Science Publ, Ann Arbour. pp 305-322.

Benjamin, M.M., Leckie, J.O.(1981) Multiple-site adsorption of Cd, Cu Zn and Pb on amorphous iron oxyhydroxide. *J. Coll. Interface Sci.* Vol **79**, pp 209-221.

Benfield, S. Guzman, H., Mair, J. (2005) Temporal mangrove dynamics in relation to coastal development in Pacific Panama. Journal of Environmental Management Vol **76**, Issue 3, pp 263-276.

Bryan, G.W., Langston, R.J. (1992) Bioavailability, accumulation and effects of heavy metals in sediments, with special reference to United Kingdom estuaries: a review. *Environmental Pollution* Vol **76**, pp 89-131.

Church, T.M., Arimoto, R., Barrie, L.A., Deharis. F., Dulac. F., Jickells, T.D., Mart. L., Sturgers. W.T., Zoller, W.H. (1990) The long-range atmospheric transport of trace elements: A critical evaluation. In: The long-range atmospheric transport of natural and contaminated substances. (Ed) Knap, A.H. Kluwer-Dordrecht, pp 37-58.

Clark, M.W., McConkie, D., Lewis, D.W., Saenger. P. (1998) Redox stratification

and heavy metal partitioning in *Avicennia*-dominated mangrove sediments: a geological model. *Chemical Geology* Vol **149**, pp 147-171.
Davies, A.G., Sleep, J.A. (1980) Copper inhibitions of carbon fixation in coastal phytoplankton assemblages. *J. Mar. Biol. Assoc*. Vol **60**, pp 841-850.

Defew, L., Mair, J., Guzman, H. (2004) An assessment of metal contamination in mangrove sediments and leaves from Punta Mala Bay, Pacific Panama. *Marine Pollution Bulletin* Vol **50**, No 5, pp 547-552.

DeGregori, I., Pinochet, H., Arancibia, M., Vidal, A. (1996) Grain Size Effects on Trace Metals Distribution in Sediments from Two Coastal Areas of Chile. *Bull. Envrin. Contam. Toxicol*. Vol **57**, pp 163-170.

Fores-Rodriguez, J., Bussy, A-L., Thevenot, D.R. (1994) Toxic metals in urban runoff: physico-chemical mobility assessment using speciation schemes. *Wat. Sci. Tech.* Vol **29**, Nos 1-2, pp 83-93.

Forstner, U. (1979) Metal transfer between solid and aqueous phases. In: Metal Pollution in the Aquatic Environment. (Ed) Forstner, U., Whittman, G.T.W. Springer-Verlag, Berlin. pp-197-270.

Fritoff, A., Kautsky, L., Greger, M. (2003) Influence of temperature and salinity on heavy metal uptake by submersed plants. *Environmental Pollution* Vol **133**, pp 265-274.

Gambrell, R.P. (1994) Trace and Toxic Metals in Wetlands – A review. *J. Envion. Qual*. Vol **23**, pp 883-891.

Gardiner, J. (1974) The chemistry of cadmium in natural waters – II. The adsorption od cadmium on river muds and naturally occurring solids. *Water Res*. Vol **8**, pp157-164.

Guzman, H., Garcia, E. (2002) Mercury levels in coral reefs along the Caribbean coast of Central America. *Marine Pollution Bulletin* Vol **44**, pp 1415-1420.

Guzman, H.M., Jackson, J.B.C., Weil, E. (1991) Short-term ecological consequences of a major oil spill on Panamanian subtidal reef corals. *Coral Reefs* Vol **10**, pp 1-12.

Guzman, H., Jarvis, K. (1996) Vanadium Century Record from Caribbean Reef Corals: A Tracer of Oil Pollution in Panama. *AMBIO, A Journal of the Human Environment* Vol **25**, No 8, pp 523-526.

Guzman, H., Jimemez, C. (1992) Contamination of coral reefs by heavy metals along the Caribbean coast of Central America. *Marine Pollution Bulletin* Vol **24**, No 11, pp 554-561.

Hansen, M.H., Ingvorsen. K., Jorgensen, B.B. (1978) Mechanisms of hydrogen sulphide release from coastal marine sediments to the atmosphere. *Limnol. Oceanogr*. Vol **23**, pp 68-76.

Harbison, P. (1986) Mangrove muds – a sink and a source for Trace Metals. *Marine Pollution Bulletin* Vol **17**, No 6, pp 246-250.

Harris, R.R., Santos, M.C.F. (2000) Heavy metal contamination and physiological variability in the Brazilian mangrove crabs *Ucides cordatus* and *Callinectes danae* (Crustacea: Decapoda). *Marine Biology* Vol **137**, pp 691-703.

Hart, B. (1982) Uptake of trace metals by sediments and suspended particulates: a review. *Hydrobiologia* Vol **91**, pp 299-313.

Hassett, J.M., Jennett, J.C, Smith, J.E. (1980) Heavy metals accumulation by algae. In: Contaminants and Sediments Vol **2**. (Ed) Baker, R. A. Ann Arbour Science Publ. Ann Arbour. Pp 409-424.

Herut, B., Hornung, H., Krom, M.D., Kress, N., Cohen, Y. (1993) Trace metals in shallow sediments from the Mediterranean coastal region of Israel. *Marine Pollution Bulletin* Vol **26**, No 12, pp 675-682.

Jickells, T. (1995) Atmospheric inputs of metals and nutrients to the oceans: their magnitude and effects. *Marine Chemistry* Vol **48**, pp 199-214.

Kendrick, M.J., May, M.T., Plishka, M.J., Robinson, K.D. (1992) *Metals in Biological Systems*. Ellis Horwood Ltd.

Koppe, D.E. (1981) Lead: Understanding the Minimal Toxicity of Lead in Plants. In: Effect of Heavy Metal Pollution on plants, Vol **I**. (Ed) Lepp, N.W. Applied Science Publishers London and New Jersey. pp 55-76.

Long, E. (1992) Ranges in chemical concentrations in sediments associated with adverse biological effects. *Marine Pollution Bulletin* Vol **24**, No 1, pp 38-45.

MacFarlane, G.R., Pulkownik, A., Burchett, M.D. (2003) Accumulation and distribution of heavy metals in the grey mangrove, *Avicennia marina* (Forsk.) Vierh: biological indication potential. *Environmental Pollution* Vol **123**, pp 139-151.

MacFarlane, G.R. (2002) Leaf biochemical parameters in Avciennia marina (Forsk.) Vierh as potential biomarkers of heavy metals stress in estuarine ecosystems. Marine Pollution Bulletin Vol 44, pp65-256.

Martin, J.H. (1990) Glacial-interglacial CO2 change: the iron hypothesis. *Palaeoceanography*. Vol **5**, pp 1-13.

Mateu, J., Forteza, M., Colom-Altes, M., Cerda. V. (1996) Atmospheric background levels and transport of heavy metals in the Balearic Islands. *Water, Air and Soil Pollution* Vol **86**, pp 157-172.

Minear, A.R., Murray, B.B. (1974) Methods of Trace Metals in Analysis in Aquatic Systems. In: Trace *Meals and Metal-Organic Interactions in Natural Waters*. (Ed) Singer, P.C. Ann Arbour Science, USA. pp 1-43.

Ong Che, R.G. (1999) Concentration of 7 Heavy Metals in Sediments and Mangrove Root Samples from Mai Po, Hong Kong. Marine Pollution Bulletin Vol **39**, Nos 1-12, pp 269-279.

Pacyna, J.M., Munch. J., Axenfeld. F. (1991) European inventory of trace metal emissions to the atmosphere. In: Heavy Metals in the Environment 1. (Ed) Vernet, J-P. Elsevier, Amsterdam, London, New York & Toyko. pp 1-20.

Perdomo, L., Ensminger, I., Espinosa, L.F., Elster, C., Wallner-Kersanach, M., Schnetter, M. (1998) The Mangrove Ecosystem of the Cienaga Grande de Santa Marta (Colombia): Observations on Regeneration and Trace Metals in Sediment. *Marine Pollution Bulletin* Vol **37**, Nos 8-12, pp 393-403.

Philips, D.A. (1981) Chemistry and Biochemistry of Trace Metlas in biological systems. In Effect of Heavy Metal Pollution on plants, Vol **I**. (Ed) Lepp, N.W. Applied Science Publishers London and New Jersey. pp 1-54.

Puckett, K.J., Burton, M.A.S. (1981) The effect of trace elements on lower plants. In Effect of Heavy Metal Pollution on plants, Vol **II**. (Ed) Lepp, N.W. Applied Science Publishers, London & New Jersey. pp 239-249.

Quevauiller, PH., Kramer, K.J.M., Van Der Vilies, E.M., Vercoutere, K., Griepink, B. (1992) Improvements in the Determination of Trace Elements in Seawater, leading to the certification of Cd, Cu, Mo, Ni, Pb and Zn (CRM 403). *Marine Pollution Bulletin* Vol **24**, No 1, pp 33-38.

Rainbow, P.S.(1995) Bio-monitoring of heavy metal availability in the marine environment. *Marine Pollution Bulletin* Vol **31** (4), pp 183-192.

Sager, M. (1997) Possible trace metal load from fertilizers. *Die Bodenkultur* Vol **48** (4), pp 217-223.

Schlinder, P.W. (1991) The regulation of heavy metal concentrations in natural aquatic systems. In Heavy Metals in the Environment 1. (Ed) Vernet, J-P. Elsevier, Amsterdam, London, New York and Toyko. pp 95-124.

Shin, P.K.S., Fong, K.Y.S. (1999) Multiple Discriminant Analysis of Marine Sediment Data. *Marine Pollution Bulletin* Vol **39**, Nos 1-12, pp 285-294.
Siedlecka, A. (1995) Some aspects of interactions between heavy metal and plant mineral nutrients. *Acta. Soc. Bio. Pol*. Vol **64** (3), pp 265-272.

Singer, P.C. (1974) Trace *Meals and Metal-Organic Interactions in Natural Waters.* Ann Arbour Science, USA.

Stumm, W., Morgan, J.J. (1981) In: Aquatic Chemistry. 2nd Edition. John Wiley & Sons Inc. New York, London, Sydney & Toronto. pp 780.

Szefer, P., Geldon, J., Ahmed Ali, A., Paez Osuna, F., Ruiz-Fernandes, A.C., Guerro Gaivan, S.R. (1998) *Environmental International* Vol 24 (3), pp 359-374.

Tam, N.F.Y., Wong,Y.S. (2000) Spatial variation of heavy metals in surface sediments of Hong Kong mangrove swamps. *Environmental Pollution* Vol **110**, pp195-205.

Thapa, G.B., Weber, K.E. (1991) Soil erosion in developing countries: a politicoeconomic explanation. *Environ. Man.* Vol **15**, pp 461-473.

Waldichuk, M. (1985) Biological availability of metals to marine organisms. *Marine Pollution Bulletin* Vol **16**, pp7-11.

Webber, J. (1981) Trace Metals in Agriculture. In: Effect of Heavy Metal Pollution on plants, Vol **II**. (Ed) Lepp, N.W. Applied Science Publishers London & New Jersey. pp 159-184.

White, D.E. (2004) A mapping study of the seagrass Zoster in the Forth Estuary using GPS and ariel photography and an assessment of their potential to accumulate trace metals. Msc Dissertation, Heriot Watt University.

Wollast, R. (1982) Methology of research in micropollutants – heavy metals. *Wat. Sci. Tech*. Vol **14**, pp 107-125.

Yim, M.W., Tam, N.F.Y. (1999) Effects of Wastewater-borne Heavy Metals on Mangrove Plants and Soil Microbial Activities. *Marine Pollution Bulletin* Vol **39**, Nos 1-12, pp 179-186.

http://workall.com/wfb2001/panam/panama_geography.html (27/07/2005)

http://www.eia.doe.gov/emu/cabs/centam.html (27/07/2005)

ATSCR http://www.atsdr.cdc.gov/HEC/CSEM/chromium/sources_exposure.html
Lenntech Heavy Metals http://www.lenntech.com/heavy-metals.htm
http://www.scienceagogo.com/news/19990109225423data_trunc_sys.shtml
http://www.lenntech.com/Periodic-chart-elements/Ni-en.htm
http://www.lenntech.com/Periodic-chart-elements/Pb-en.htm
http://www.lenntech.com/Periodic-chart-elements/Mn-en.htm
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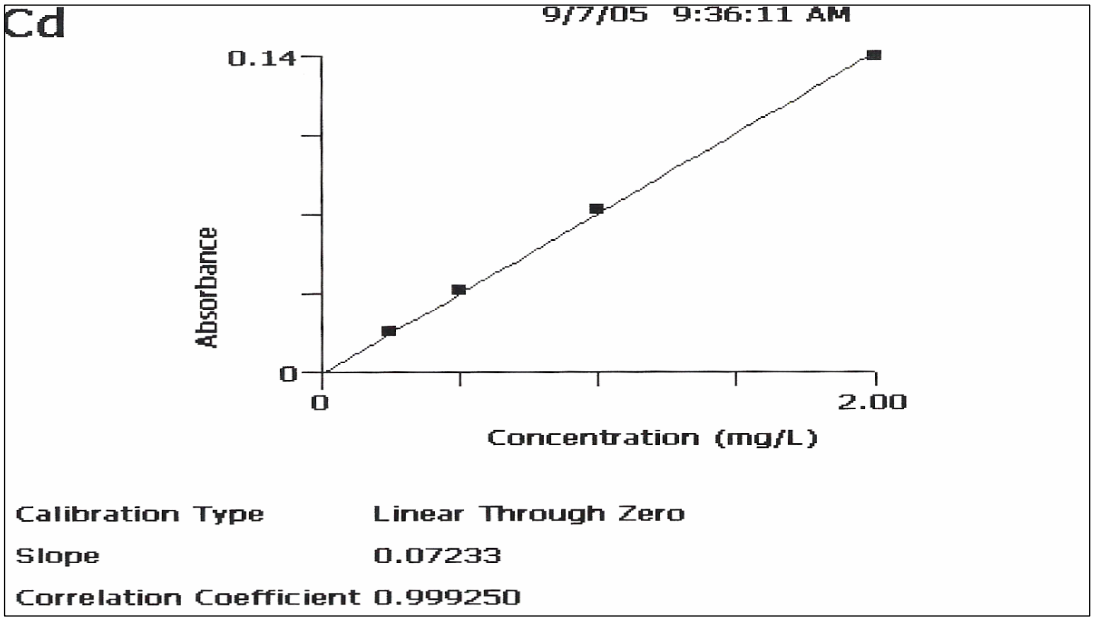
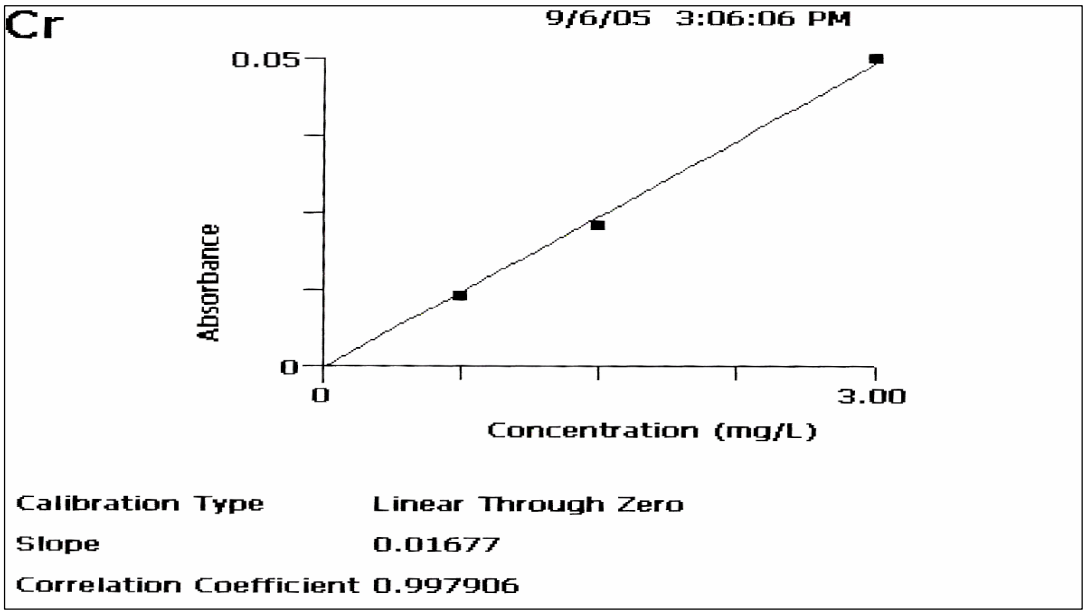
http://www.lenntech.com/Periodic-chart-elements/Cu-en.htm

(All accessed on 30/07/2005)

Appendix 1. CALIBRATION CURVES

Displayed below are all the calibration curves used to calibrate the Atomic Absorption Spectrometer for each specific metal.

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Absorbance
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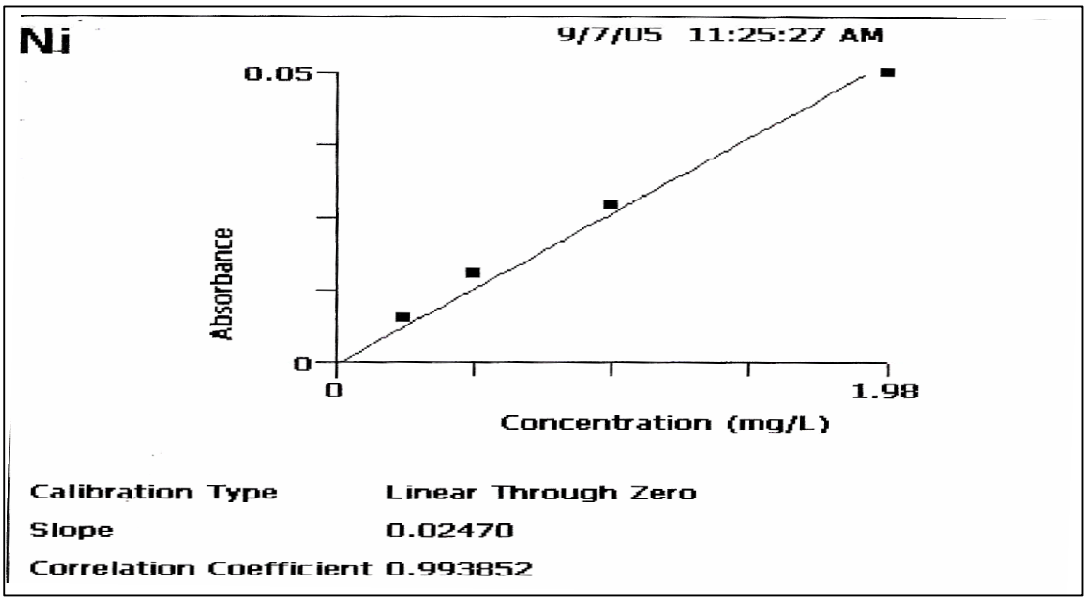
3.00
Concentration (mg/L)
Calibration Type Linear Through Zero
Slope 0.01677
Correlation Coefficient 0.997906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Absorbance
0
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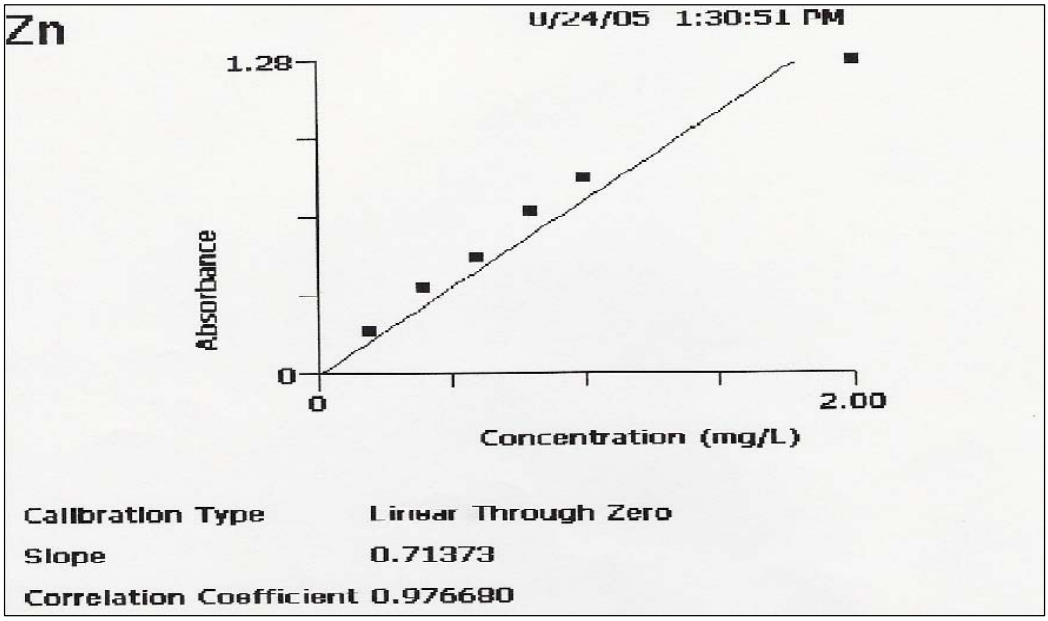
Concentration (mg/L)
Calibration Type Linear Through Zero
Slope 0.02470
Correlation Coefficient 0.993852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Absorbance
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Concentration (mg/L)
Calibration Type Linear Through Zero
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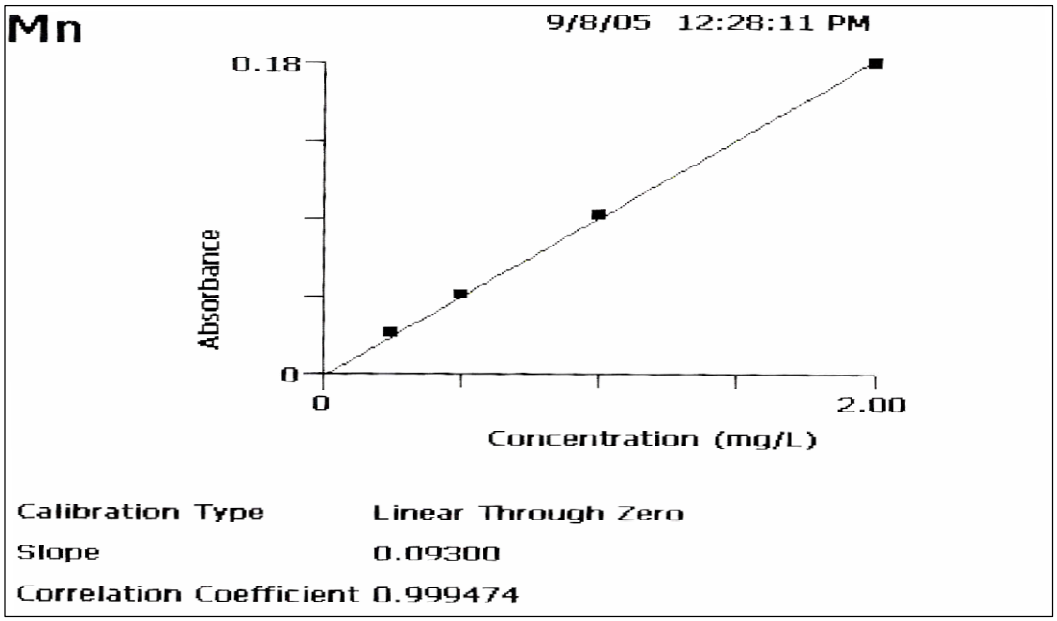
Correlation Coefficient 0.976680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2.00
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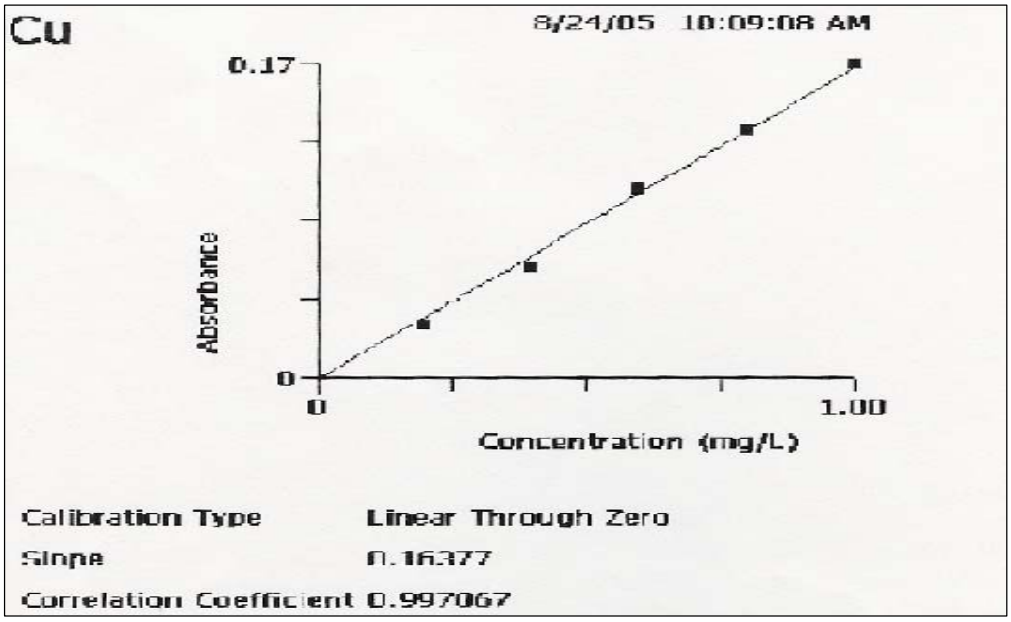
Calibration Type Linear Through Zero
Slope 0.09300
Correlation Coefficient 0.999474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1.00
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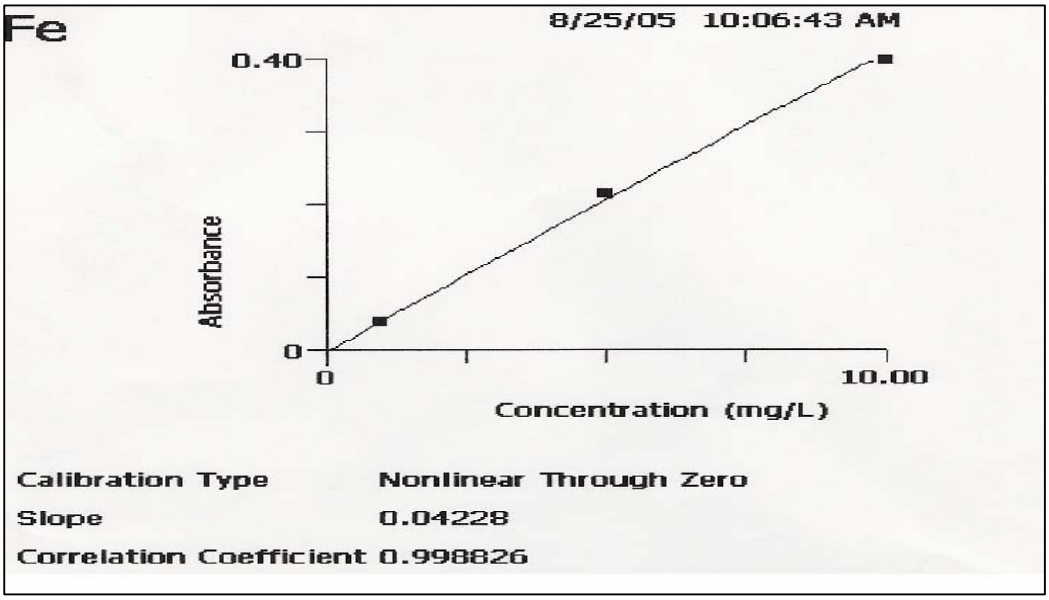
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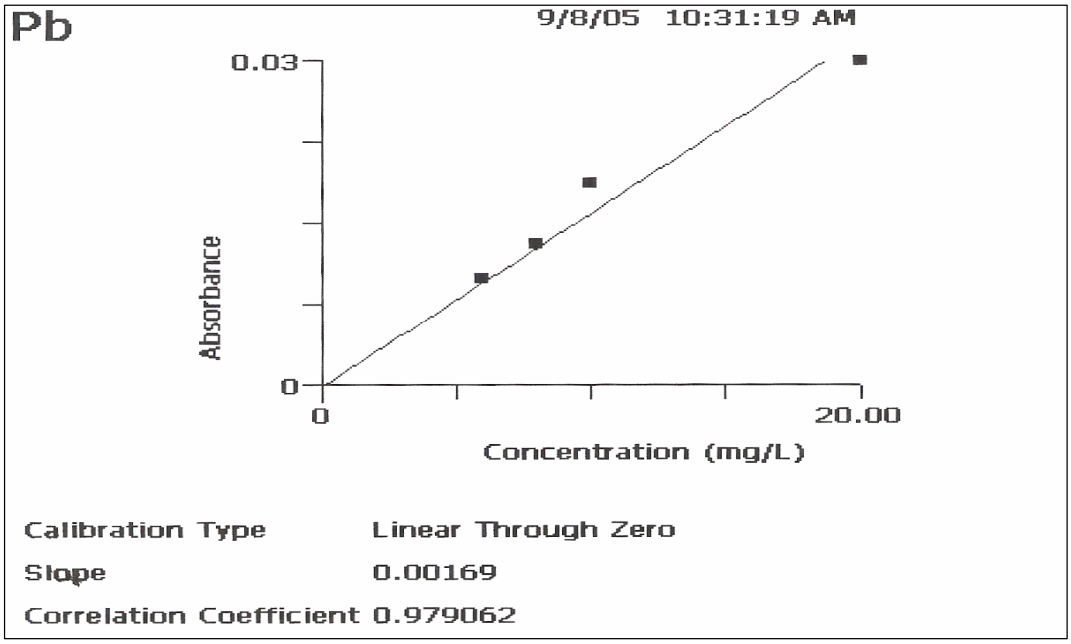

Appendix 2. **RESULTS AND CONCENTRATIONS**

Zn	Absorbance	RSD	Concentrarion
		%	mg / kg
K1-1	1.839	0.2	71.83
-2	1.878	1.3	73.35
K2-1	1.107	0.3	43.2
-2	1.297	0.1	50.66
K3-1	1.436	0.5	56.09
-2	1.331	0.2	51.99
K4-1	1.453	0.7	56.75
-2	1.317	0.6	51.45
K5-1	0.697	0.1	27.23
-2	1.021	0.6	65.35
K6-1	0.683	0.3	26.68
-2	1.278	0.4	49.9
K7-1	1.41	0.2	55.08
-2	1.494	0.1	58.36
K8-1	0.3	0.4	23
-2	0.301	0.5	23.5
K9-1	0.257	0.4	20.07
-2	0.25	0.7	19.53
K10-1	1.67	0.6	130.46
-2	1.658	0.4	129.53
K11-1	1.406	0.4	109.84

-2	1.472	0.4	115
K12-1	0.745	0.4	58.2
-2	0.562	0.5	43.9
K13-1	1.354	0.6	52.89
-2	1.527	0.4	59.64
K14-1	1.115	0.3	43.55
-2	0.843	0.4	32.92
K15 -1	1.368	0.8	53.43
-2	1.279	1.1	49.96
K16-1	1.354	0.9	52.9
-2	0.933	0.3	36.44
K17-1	0.191	0.7	14.92
-2	0.283	0.5	22.11
K18-1	1.069	0.9	41.75
-2	1.219	0.1	47.62
K19-1	0.645	0.2	25.2
-2	1.243	0.9	48.55
K20-1	0.17	1.7	13.28
-2	0.151	0.6	19.33
K21-1	0.183	0.9	14.3
-2	0.174	0.3	13.5
K22-1	0.67	1	52.34
-2	0.636	0.5	49.7
K23-1	0.18	1.1	14.06
-2	0.159	0.4	12.42
K24-1	0.304	0.3	23.75
-2	0.309	0.5	24.14
K25 -1	0.146	0.5	11.4
-2	0.519	0.8	40.546
K 26 -1	1.081	0.2	84.45
-2	1.157	0.4	90.39
K36 -1	0.909	0.5	71.02
-2	0.879	0.2	68.67
K37 -1	1.569	0.4	122.58
-2	1.494	0.4	116.72
K38 -1	1.069	0.1	83.5
-2	1.333	0.5	104.14
K39 -1	1.729	0.7	135.08
-2	1.686	0.3	131.72
T2 -1	1.526	1.2	59.6
-2	1.45	0.2	56.64
T3 -1	1.233	0.1	48.16
-2	1.574	0.1	49.77
T4 -1	1.591	0.2	62.15
-2	1.217	0.3	47.54
T5 -1	1.271	0.5	49.65
-2	1.481	0.2	57.85
T6 -1	1.404	0.3	54.84
-2	1.512	0.6	59.06
T7 -1	1.364	0.9	53.28
-2	1.411	0.4	55.11
T8 -1	1.514	0.4	59.14

-2	1.552	0.9	60.63
T9 -1	1.584	0.7	61.88
-2	1.438	0.1	56.17
T10 -1	1.437	1	56.13
-2	1.289	0.2	50.35
A-1	0.885	0.7	34.57
-2	0.263	3.1	10.27
B-1	0.718	0.4	28.05
-2	0.778	0.4	30.39
C-1	0.781	0.9	30.51
-2	0.609	0.3	23.79
D-1	0.671	0.4	26.21
-2	0.722	0.7	28.2
E-1	0.796	0.2	31.1
-2	0.7	0.2	27.34
F-1	0.963	0.3	37.62
-2	1.073	0.3	41.91
G-1	0.535	0.7	20.9
-2	0.76	0.4	29.69
H-1	0.544	1	21.25
-2	0.63	0.6	24.61
I-1	0.639	0.7	24.96
-2	0.672	0.7	26.25
J-1	1.315	0.3	51.37
-2	1.289	0.3	50.35
K-1	0.224	1.4	8.75
-2	0.237	1.2	9.26
4-I	0.456	0.9	17.81
-2	0.296	1.1	11.56
9-I	0.703	0.3	27.46
-2	0.216	2.5	8.4
26-I	0.568	0.1	22.19
-2	0.431	0.3	16.83
30-I	0.782	0.6	30.5
-2	0.742	0.5	28.98
38-I	0.17	1.3	6.64
-2	0.161	1.7	6.29
47-I	0.555	0.3	21.68
-2	0.49	0.1	19.14
62-I	0.618	0.4	24.14
-2	0.626	0.6	24.45
67-I	0.548	0.2	21.4
-2	0.561	0.7	21.91
92-I	0.224	1.7	8.75
-2	0.188	1	7.34
95-I	0.53	2	20.7
-2	0.55	1	21.48
112-I	0.168	1.3	6.56
-2	0.119	4.2	4.65

Fe	Concentration	RSD	1 in	150	1 in	300	1 in	600	Concentration
	PPM	%	PPM	%	PPM	%	PPM	%	mg / kg
K1-1	31.671	0.7	14.697	0.3	7.784	0.2			58380
-2	31.519	0.3	13.159	0.5	7.178	0.5			53835
K2-1	27.467	0.3	4	0.9					15000
-2	26.584	1	5.487	0.9					20576.25
K3-1	26.328	1.4	17.643	0.4	5.945	84.7			44587.5
-2	28.98	0.5	23.357	0.2	13.63	0.4	10.683	0.4	160245
K4-1	9.691	1.7							242.27
-2	9.517	1.4							237.92
K5-1	8.143	1.7							203.57
-2	7.094	0.9							179.85
K6-1	8.283	0.6							207.07
-2	8.351	0.4							208.77
K7-1	9.426	2.2							235.65
-2	9.561	1.8							239.02
K8-1	1.978	8.9							98.9
-2	5.34	1							267
K9-1	1.567	0.7							78.35
-2	2.005	0.82							100.25
K10-1	8.217	1							410.85
-2	56.827	1.7							341.35
K11-1	7.571	1.6							378.55
-2	7.869	0.3							393.45
K12-1	8.259	0.9							412.95
-2	7.756	2.5							387.8
K13-1	9.706	2							242.65
-2	9.754	1.6							243.85
K14-1	5.164	1.7							129.1
-2	5.726	1							143.15
K15 -1	9.062	0.8							226.55
-2	8.659	3							216.47
K16-1	7.738	1.1							193.45
-2	6.531	1.6							163.275
K17-1	1.453	24.1							72.65
-2	3.184	3.1							159.2
K18-1	9.015	2.1							450.75
-2	9.287	2							464.35
K19-1	8.188	2							409.4
-2	7.498	1.2							374.9
K20-1	5.861	5.4							284.05
-2	3.042	4.4							152.1
K21-1	1.277	2.4							63.85
-2	1.335	6.2							66.75
K22-1	8.246	1.3							412.3
-2	8.111	2							405.55
K23-1	0.342	35.9							17.1
-2	0.112	69							5.6
K24-1	5.028	0.8							251.4
-2	5.248	4.7							262.4

K25 -1	2.393	7.7		119.65
-2	0.277	27		13.85
K 26 -1	9.743	1.5		487.15
-2	9.241	2.4		462.05
K36 -1	8.498	0.5		424.9
-2	8.664	1.4		433.2
K37 -1	7.804	0.7		390.2
-2	7.913	1.8		395.65
K38 -1	7.187	3.8		359.35
-2	8.025	3		401.25
K39 -1	8.687	2.1		434.35
-2	8.468	1.2		423.4
T2 -1	8.901	1.1		222.52
-2	8.62	3.4		215.5
T3 -1	9.352	1.9		233.8
-2	9.137	2.1		228.42
T4 -1	9.081	1.5		227.02
-2	9.139	1.1		228.47
T5 -1	9.33	3.2		233.25
-2	9.47	0.9		236.75
T6 -1	9.682	1.8		242.05
-2	9.329	2.9		233.22
T7 -1	9.292	0.8		232.3
-2	9.269	1.9		231.72
T8 -1	8.714	2.1		217.85
-2	8.938	2.2		223.45
T9 -1	9.299	3.6		232.47
-2	9.749	2.2		243.72
T10 -1	8.774	1.7		219.35
-2	8.541	3.7		213.52
A-1	4.701	1.7		117.52
-2	4.91	0.7		122.75
B-1	5.694	1.6		142.35
-2	5.809	2.1		145.22
C-1	4.728	3.1		118.2
-2	1.736	1.8		43.4
D-1	4.112	2		102.8
-2	4.154	1.5		103.85
E-1	9.504	2.6		237.6
-2	9.04	2.4		226
F-1	8.337	0.9		208.42
-2	8.055	1.4		201.37
G-1	5.052	4		126.3
-2	5.199	1.2		129.97
H-1	2.329	3.1		58.22
-2	4.742	2.8		118.55
I-1	9.154	2.2		228.85
-2	9.067	1.1		226.67
J-1	9.833	1		245.82
-2	9.53	3.3		238.25
K-1	2.238	11.4		55.95
-2	4.575	3.7		114.37

4-I	8.437	0.9		210.92
-2	7.565	0.4		189.12
9-I	6.905	2		172.62
-2	7.707	3.7		192.67
26-I	8.231	1.9		205.77
-2	7.656	1.7		191.4
30-I	8.926	1.1		223.15
-2	8.872	1.4		221.8
38-I	5.419	2.1		135.47
-2	5.749	4.2		143.72
47-I	9.575	1.1		239.37
-2	9.683	1.2		242.07
62-I	8.808	1.4		220.2
-2	9.149	4.4		228.72
67-I	10.346	2.4		258.65
-2	10.112	2.9		252.8
92-I	6.448	2.3		161.2
-2	6.773	1.3		169.32
95-I	9.158	0.8		228.95
-2	9.262	2		231.55
112-I	7.538	0.2		188.45
-2	6.013	3.7		150.32

Ni	Mean Concentration	RSD	Concentration
	PPM	%	mg / kg
K1-1	0.66	1.8	16.5
-2	0.73	0.4	18.25
K2-1	0.64	3.5	16
-2	0.61	3	15.25
K3-1	0.86	1.3	21.5
-2	0.85	1.2	21.45
K4-1	0.52	1.1	13
-2	0.51	3.2	12.75
K5-1	0.46	0.8	11.5
-2	0.34	1.6	8.5
K6-1	0.42	1.2	10.5
-2	0.45	6.2	11.25
K7-1	0.49	6.1	12.25
-2	0.47	5.7	11.75
K8-1	0.37	2	18.5
-2	0.39	5.8	19.5
K9-1	0.42	0.45	21
-2	0.12	2.2	6
K10-1	0.29	3	14.5
-2	0.36	1.7	18
K11-1	0.31	6.6	15.5
-2	0.3	4.6	15
K12-1	0.32	5	16
-2	0.32	4.4	16
K13-1	0.74	1.5	18.5
-2	0.75	1.5	18.75
K14-1	0.48	3.8	12
-2	0.5	1.7	12.5
K15 -1	0.7	2.2	17.5
-2	0.6	2.1	15
K16-1	0.64	2.3	16
-2	0.42	5.3	10.5
K17-1	0.34	5.3	17
-2	0.28	1.7	14
K18-1	0.43	3	10.75
-2	0.47	1.8	11.75
K19-1	0.53	0.8	13.25
-2	0.45	2.9	11.25
K20-1	0.35	0.9	17.5
-2	0.27	11	13.5
K21-1	0.28	3.6	14
-2	0.27	6	13.5
K22-1	0.49	3	24.5
-2	0.44	1.5	22
K23-1	0.39	12.9	19.5
-2	0.46	3.1	23
K24-1	0.35	4.5	17.5

-2	0.32	21.3	16
K25 -1	0.38	5.6	19
-2	0.38	3.8	19
K 26 -1	0.73	3.7	36.5
-2	0.71	2.7	35.5
K36 -1	0.29	10.3	14.5
-2	0.3	3.1	15
K37 -1	0.39	12.7	19.5
-2	0.33	10	16.5
K38 -1	0.11	52.1	5.5
-2	0.32	8.3	16
K39 -1	0.38	3.5	19
-2	0.45	12.3	22.5
T2 -1	0.4	10.4	10
-2	0.62	3.7	15.5
T3 -1	0.57	4.4	14.25
-2	0.67	4.8	16.75
T4 -1	0.73	3.5	18.25
-2	0.56	4.8	14
T5 -1	0.59	3.3	14.75
-2	0.54	5.8	13.5
T6 -1	0.52	1.4	13
-2	0.52	0.8	13
T7 -1	0.45	0.6	11.25
-2	0.47	8.4	11.75
T8 -1	0.39	3.9	9.75
-2	0.49	0.8	12.25
T9 -1	0.47	4.5	11.75
-2	0.48	2.2	12
T10 -1	0.31	14.6	7.75
-2	0.34	12	17
A-1	0.49	4	12.25
-2	0.52	5.8	13
B-1	0.5	2.1	12.5
-2	0.55	8.6	13.75
C-1	0.46	11.8	1.5
-2	0.52	5.1	13
D-1	0.54	3.1	13.5
-2	0.46	10.8	11.5
E-1	0.66	3.5	16.5
-2	0.29	18.4	7.25
F-1	0.28	19.4	7
-2	0.39	4.8	9.75
G-1	0.33	3.9	8.25
-2	0.4	9.8	10
H-1	0.28	19.5	7
-2	0.28	11	7
I-1	0.3	14.1	7.5
-2	0.31	3.5	7.75
J-1	0.31	14.3	7.75
-2	0.47	2.4	11.75
K-1	0.34	14.7	8.5

-2	0.38	9.7	9.5
4-I	0.6	5	15
-2	0.5	3.2	12.5
9-I	0.32	19.7	8
-2	0.51	7.7	12.75
26-I	0.38	9.8	9.5
-2	0.25	19.9	6.25
30-I	0.57	3	14.25
-2	0.57	10	14.25
38-I	0.31	22.6	7.75
-2	0.24	25.4	6
47-I	0.27	14.4	6.75
-2	0.28	19.8	7
62-I	0.46	8.9	11.5
-2	0.53	14.7	13.25
67-I	0.3	23.4	7.5
-2	0.33	8.5	8.25
92-I	0.35	4.4	12.25
-2	0.27	15.3	6.75
95-I	0.49	14	12.25
-2	0.44	8.9	11
112-I	1.18	3.1	29.5
-2	0.27	26.9	6.75

Cd	Mean Concentration	RSD	Concentration
	PPM	%	mg / kg
K1-1	0.21	2.2	5.25
-2	0.21	3.8	5.25
K2-1	0.23	0.6	5.75
-2	0.23	0.3	5.75
K3-1	0.14	3	3.5
-2	0.15	0.7	3.75
K4-1	0.12	4	3
-2	0.12	2.1	3
K5-1	0.12	0.8	3
-2	0.11	3.3	2.75
K6-1	0.04	6	1
-2	0.04	6.5	1
K7-1	0.06	2	1.5
-2	0.06	7.1	1.5
K8-1	0.11	2.5	5.5
-2	0.1	4.1	5
K9-1	0.11	2	5.5
-2	0.21	2.2	10.5
K10-1	0.05	12.6	2.5
-2	0.04	3.9	2
K11-1	0.05	2.1	2.5
-2	0.05	5.7	2.5
K12-1	0.04	7.4	2
-2	0.06	3.7	3
K13-1	0.08	1.6	2
-2	0.07	1.1	1.75
K14-1	0.12	4.8	3
-2	0.15	1.7	3.75
K15 -1	0.1	2	2.5
-2	0.1	1.6	2.5
K16-1	0.18	3.6	4.5
-2	0.12	1.2	3
K17-1	0.13	0.4	6.5
-2	0.13	0.4	6.5
K18-1	0.09	2.2	4.5
-2	0.09	7.7	4.5
K19-1	0.15	3	7.5
-2	0.14	1.9	7
K20-1	0.16	1.4	8
-2	0.15	3.9	7.5
K21-1	0.11	5	5.5
-2	0.11	1.8	5.5
K22-1	0.1	4.8	5
-2	0.1	1.9	5
K23-1	0.13	1.2	6.5
-2	0.19	4.3	9.5
K24-1	0.13	3.1	6.5

-2	0.13	2.4	6.5
K25 -1	0.16	4.1	8
-2	0.15	2.4	7.5
K 26 -1	0.11	5.7	5.5
-2	0.11	3.9	5.5
K36 -1	0.1	4.5	5
-2	0.09	6.5	4.5
K37 -1	0.11	5.1	5.5
-2	0.12	3.3	6
K38 -1	0.13	2.9	6.5
-2	0.11	1.6	5.5
K39 -1	0.12	5.5	6
-2	0.12	5.3	6
T2 -1	0.06	6.1	1.5
-2	0.07	7.6	1.75
T3 -1	0.06	8	1.5
-2	0.07	9.1	1.75
T4 -1	0.06	6	1.5
-2	0.06	4.1	1.5
T5 -1	0.05	0.7	1.25
-2	0.05	4.2	1.25
T6 -1	0.06	9.1	1.5
-2	0.07	0.7	1.75
T7 -1	0.07	1.5	1.75
-2	0.07	1.8	1.75
T8 -1	0.08	7.6	2
-2	0.08	1.8	2
T9 -1	0.11	1.3	2.75
-2	0.11	2.4	2.75
T10 -1	0.09	0.7	2.25
-2	0.09	8.4	2.25
A-1	0.13	4.5	3.25
-2	0.15	1.9	3.75
B-1	0.15	4.7	3.75
-2	0.16	1	4
C-1	0.16	1.7	4
-2	0.15	4.3	3.75
D-1	0.17	4.2	4.25
-2	0.16	6	4
E-1	0.07	4.2	1.75
-2	0.05	15.6	1.25
F-1	0.02	48.2	0.5
-2	0.06	1.9	1.5
G-1	0.14	1.8	3.5
-2	0.14	4.8	3.5
H-1	0.13	4.2	3.25
-2	0.15	5.7	3.75
I-1	0.05	18	1.25
-2	0.05	9.9	1.25
J-1	0.03	6.7	0.75
-2	0.03	34.4	0.75
K-1	0.11	5.1	2.75

-2	0.1	16.8	2.5
4-I	0.08	11.6	2
-2	0.08	18.4	2
9-I	0.05	16.9	1.25
-2	0.07	30.1	1.75
26-I	0.03	34.6	0.75
-2	0.06	12.6	1.5
30-I	0.09	15.9	2.25
-2	0.09	1.2	2.25
38-I	0.12	2.2	3
-2	0.13	4.8	3.25
47-I	0.04	13.7	1
-2	0.05	10	1.25
62-I	0.01	52.5	0.25
-2	0.06	5.6	1.5
67-I	0.06	14.1	1.5
-2	0.06	14.4	1.5
92-I	0.14	0.4	3.5
-2	0.13	7.9	3.25
95-I	0.03	27.4	0.75
-2	0.03	22.8	0.75
112-I	9	3.6	2.25
-2	0.09	5.4	2.25

Pb	Mean Concentration	RSD	Concentration
	PPM	%	mg / kg
K1-1	0.23	1272.1	5.75
-2	0.42	338.9	10.5
K2-1	3.16	43.8	79
-2	0.57	197.9	14.25
K3-1	0.5	294.8	12.5
-2	0.23	304.4	5.75
K4-1	0.03	6545.1	0.75
-2	0.63	263	15.75
K5-1	1.88	112.4	47
-2	0.68	411.3	17
K6-1	1.66	82.9	41.5
-2	1	72.4	25
K7-1	0.46	212.6	11.5
-2	2.01	60.6	50.25
K8-1	0.7	195.1	35
-2	1.25	31.3	64
K9-1	1.4	56.4	70
-2	2.7	3.3	135
K10-1	3.06	88.4	153
-2	3.53	26.3	176.5
K11-1	4.27	33.6	213.5
-2	0.74	166.7	37
K12-1	0.42	239.6	21

-2	1.92	96.8	96
K13-1	1.01	183.5	25.25
-2	4.1	36.6	102.5
K14-1	3.35	68.8	83.75
-2	3.36	55.9	84
K15 -1	6.21	13.7	155.25
-2	3.42	43.1	85.5
K16-1	6.28	15.9	157
-2	5.85	35.7	146.25
K17-1	6.5	5.1	325
-2	7.84	11.7	392
K18-1	5.29	21.6	132.25
-2	6.78	15.1	169.5
K19-1	4.36	40.6	109
-2	5.63	12.3	140.75
K20-1	5.66	12.8	283
-2	6.75	32.3	337.5
K21-1	5.23	30.1	261.5
-2	6.21	25.5	310.5
K22-1	5.47	10.3	273.5
-2	6.77	6.4	338.5
K23-1	4.9	28	245
-2	4.48	32	224
K24-1	5.39	40.5	269.5
-2	5.7	33	285
K25 -1	3.56	16.2	178
-2	3.78	65.4	189
K 26 -1	5.38	16.1	269
-2	6.07	25.6	303.5
K36 -1	5.69	15.4	284.5
-2	4.48	16.5	224
K37 -1	6.76	10.5	338
-2	8.15	31.3	407.5
K38 -1	5.93	27.1	296.5
-2	7.48	9.6	374
K39 -1	8.87	17.8	443.5
-2	6.28	49.1	314
T2 -1	6.84	24	171
-2	7.02	32	175.5
T3 -1	6.22	41.7	155.5
-2	5.76	26.1	144
T4 -1	7.45	18.1	186.25
-2	2.38	16.6	59.5
T5 -1	7.51	31.7	187.75
-2	6.31	20.6	157.75
T6 -1	6.18	25.2	154.5
-2	5.19	24.8	129.75
T7 -1	5.61	25.7	140.25
-2	5.26	14.4	131.5
T8 -1	7.41	45.6	185.25
-2	6.75	27.4	168.75
T9 -1	5.57	16.9	139.25

-2	5.15	29.2	128.75
T10 -1	4.32	47	108
-2	5.4	44.5	135
A-1	4.93	35	123.25
-2	5.19	86.6	129.75
B-1	3.72	42.7	93
-2	2.83	52.6	70.75
C-1	5.72	32.7	143
-2	3.19	33.9	79.75
D-1	4.01	51.7	100.25
-2	3.81	54.7	95.25
E-1	4.94	14.4	123.5
-2	3.84	41.6	96
F-1	5.18	29.8	129.5
-2	4.74	19.3	118.5
G-1	3.03	69.4	75.75
-2	3.16	19.5	79
H-1	3.58	58.9	89.5
-2	3.6	44.7	90
I-1	3.1	119.9	77.5
-2	4.76	77.1	119
J-1	5.53	70.7	138.25
-2	3.49	37.1	87.25
K-1	2.56	59.6	64
-2	3.65	56.8	91.25
4-I	2.43	81.9	60.75
-2	3.93	53.6	98.25
9-I	2.98	34.1	74.5
-2	3.21	71	80.25
26-I	4.78	36.9	119.5
-2	2.9	64.8	72.5
30-I	3.66	32.4	91.5
-2	4.23	27.3	105.75
38-I	3.13	64.9	78.25
-2	2.74	89.1	68.5
47-I	4.66	31.2	116.5
-2	3.34	19	83.5
62-I	5.07	7.7	126.75
-2	5.11	20.6	127.75
67-I	3.91	23.8	97.75
-2	5	48.6	125
92-I	2.74	44.6	68.5
-2	5.17	15.8	129.25
95-I	7.13	26.9	178.25
-2	3.2	154	80
112-I	4.15	54.7	103.75
-2	3.11	4.2	77.75

Mn	Mean Concentration	RSD	Dilutions	1 in 10	Concentration
	PPM	%	PPM	%	mg / kg
K1-1	1.7	0.4			42.5
-2	0.02	25.9			0.5
K2-1	1.08	1.1			27
-2	1.04	8.7			26
K3-1	1.27	2.6			31.75
-2	1.27	9.9			31.75
K4-1	0.84	10.7			21
-2	0.74	3.6			18.5
K5-1	0.31	94			7.75
-2	0.43	2.4			10.75
K6-1	0.01	30.8			0.25
-2	0.01	40.3			0.25
K7-1	0.01	80.6			0.25
-2	0.01	18.3			0.25
K8-1	0.37	0.6			18.5
-2	0.29	3			14.5
K9-1	0.36	2.5			18
-2	0.35	4.1			17.5
K10-1	0.36	1.8			18
-2	0.38	2.7			19
K11-1	0.07	207.3			3.5
-2	0.07	99.4			3.5
K12-1	0.12	2.1			6
-2	2.01	0.7			100.5
K13-1	~~2.93~~	~~1.6~~	0.51	0.6	127.5
-2	~~2.55~~	~~3.6~~	0.46	1	115
K14-1	0.67	9.8			16.75
-2	0.73	1.4			18.25
K15 -1	2.13	0.9			53.25
-2	1.49	1.4			37.25
K16-1	1.27	3.4			31.75
-2	0.77	3.6			19.25
K17-1	0.45	0.6			22.5
-2	0.31	0.8			15.5
K18-1	~~2.43~~	~~0.9~~	0.47	0.8	117.5
-2	~~2.88~~	~~0.4~~	0.5	1.8	112
K19-1	~~3.49~~	~~0.7~~	0.61	0.5	152.5
-2	~~2.52~~	~~0.6~~	0.45	1.2	112.5
K20-1	1.38	0.5			69
-2	1.53	0.2			76.5
K21-1	1.52	0.6			76
-2	1.54	1.4			77
K22-1	2.19	0.4			109.5
-2	~~2.96~~	~~0.6~~	0.4	0.6	1480
K23-1	1.56	0.7			78
-2	1.78	0.3			89
K24-1	0.01	166.6			0.5

-2	0.02	21.9			1
K25 -1	0.46	3.1			23
-2	0.04	4.1			2
K 26 -1	0.03	16.2			1.5
-2	0.03	6.6			1.5
K36 -1	0.03	4.7			1.5
-2	0.04	8.2			2
K37 -1	3.22	0.4	0.4	13.1	200
-2	4.73	0.4	0.38	3.8	190
K38 -1	3.99	0.9	0.41	0.4	205
-2	4.37	0.2	0.43	1.4	215
K39 -1	5.56	0.3	0.56	13	280
-2	4.15	1.3	0.52	2.3	260
T2 -1	1.94	9.1			48.5
-2	0.98	6			24.5
T3 -1	0.57	1.4			14.25
-2	0.46	2.2			11.5
T4 -1	0.52	1.7			13
-2	0.55	3.9			13.75
T5 -1	0.64	0.5			16
-2	0.51	14.3			12.75
T6 -1	6.28	0.5			158
-2	6.94	0.8			173.5
T7 -1	7.35	0.6	1.12	2.8	280
-2	6.84	0.2	0.76	1.5	190
T8 -1	6.35	0.2			158.75
-2	6.68	0.2	0.86	0.5	215
T9 -1	6.59	0.4			164.75
-2	13.04	0.3	1.73	1.7	432.5
T10 -1	12.22	0.3	1.37	1.6	342.5
-2	12.37	0.3	1.33	0.9	332.5
A-1	1.55	0.1			38.75
-2	1.71	0.2			42.75
B-1	1.71	0.7			42.75
-2	1.92	0.3			48
C-1	0.57	0.5			14.25
-2	0.63	26.9			15.75
D-1	0.68	0.9			17
-2	0.58	0.8			14.5
E-1	5.68	0.1			142
-2	5.11	0.6			127.75
F-1	3.13	0.4			78.25
-2	3.77	0.6			94.25
G-1	1.32	1.3			33
-2	1.33	0.6			33.25
H-1	0.87	0.1			21.75
-2	1.05	1			26.25
I-1	5.96	0.4			149
-2	6.48	0.3			162
J-1	5.87	0.1			146.75
-2	5.19	0.5			129.75
K-1	1.01	0.8			25.25

-2	1.34	0.3			33.5
4-I	8.25	0.3	0.81	1.1	202.5
-2	6.37	0.3			159.25
9-I	6.28	1			157
-2	2.7	5.4			67.5
26-I	5.24	4.8			131
-2	4.41	0.2			110.25
30-I	3.05	3.3			76.25
-2	3.16	1.8			79
38-I	1.88	47			94
-2	1.34	0.5			33.5
47-I	2.46	0.5			61.5
-2	2.43	0.3			60.75
62-I	4.35	0.1			108.75
-2	4.18	0.7			104.5
67-I	4.45	0.9			111.25
-2	4.47	0.1			111.75
92-I	1.58	0.2			39.5
-2	1.57	0.3			39.25
95-I	3.44	0.1			86
-2	3.4	0.4			85
112-I	1.89	0.8			47.25
-2	1.35	0.9			33.75

Cr	Mean Concentration	RSD	Concentration
	PPM	%	mg / kg
K1-1	0.39	3.5	9.75
-2	0.43	2.4	10.75
K2-1	0.4	3.3	10
-2	0.45	3.8	11.25
K3-1	0.58	3.3	14.5
-2	0.62	1.9	15.5
K4-1	0.82	2.7	20.5
-2	0.78	1.4	19.5
K5-1	0.63	1.1	15.75
-2	0.5	2	12.5
K6-1	0.51	0.6	12.75
-2	0.56	2.1	14
K7-1	0.72	1.2	18
-2	0.85	0.6	21.25
K8-1	0.51	4.1	22.5
-2	0.56	4.9	28
K9-1	0.48	2.3	24
-2	0.42	3.2	21
K10-1	0.55	2.7	27.5
-2	0.55	2.6	27.5
K11-1	0.51	2.7	25.5
-2	0.52	0.9	26
K12-1	0.82	4.2	41
-2	0.32	3	16
K13-1	1.35	1	33.75
-2	1.29	0.8	32.25
K14-1	0.54	0.9	13.5
-2	0.57	1	14.25
K15 -1	1.05	2.1	26.25
-2	0.93	0.7	23.25
K16-1	0.83	2.5	20.75
-2	0.65	1.7	16.25
K17-1	0.46	1.7	23
-2	0.44	5.2	22
K18-1	0.86	1.4	21.5
-2	0.9	1.4	22.5
K19-1	0.73	2.9	18.25
-2	0.68	1	15.75
K20-1	0.45	2.8	22.5
-2	0.56	1.8	30
K21-1	0.38	2.7	19
-2	0.37	1.6	18.5
K22-1	0.72	0.9	36
-2	0.7	2	35
K23-1	0.41	5.7	20.5
-2	0.45	6	22.5
K24-1	0.42	2.7	21

-2	0.43	5.7	21.5
K25 -1	0.4	7.5	20
-2	0.38	1.4	19
K 26 -1	1.22	2	61
-2	1.2	1	60
K36 -1	0.43	3.6	21.5
-2	0.4	1.2	20
K37 -1	0.5	2.9	25
-2	0.44	1.4	22
K38 -1	0.38	7.2	19
-2	0.49	1.8	24.5
K39 -1	0.75	2	37.5
-2	0.78	3.5	39
T2 -1	1.25	1.2	31.25
-2	1.18	2	29.5
T3 -1	1.57	1.2	39.25
-2	1.64	1.2	41
T4 -1	1.39	1	34.75
-2	1.18	0.8	29.5
T5 -1	0.98	1.6	24.5
-2	1	1.1	25
T6 -1	0.96	1.2	24
-2	1.03	2.6	25.75
T7 -1	0.83	1.2	20.75
-2	0.75	0.8	18.75
T8 -1	0.64	3.4	16
-2	0.65	3.5	16.25
T9 -1	0.31	6.5	7.75
-2	0.25	6.1	6.25
T10 -1	0.09	5.4	2.25
-2	0.04	101.8	1
A-1	-0.97	3	24.25
-2	-0.98	1.5	24.5
B-1	-0.98	1.5	24.5
-2	-0.96	0.9	24
C-1	-1.09	1.7	27.25
-2	-1.06	1.1	26.5
D-1	-0.99	1.1	24.75
-2	-1.09	0.8	27.25
E-1	-0.65	3.4	16.25
-2	-0.73	1.5	18.25
F-1	-0.94	1.9	23.5
-2	-1.03	1.9	25.75
G-1	-0.98	2.4	24.5
-2	-0.98	2.4	24.5
H-1	-1	2.2	25
-2	0.39	6.8	9.75
I-1	0.58	1.7	14.5
-2	0.61	3.4	15.25
J-1	0.9	0.6	2.5
-2	0.8	0.6	20
K-1	0.25	3.1	6.25

-2	0.19	8.7	4.75
4-I	0.62	3.4	15.5
-2	0.47	3.4	11.75
9-I	0.45	4.9	11.25
-2	0.7	2.4	17.5
26-I	0.38	7.4	9.5
-2	0.3	2.8	7.5
30-I	0.66	3	16.5
-2	0.68	1.7	17
38-I	0.24	2.7	6
-2	0.25	13.5	6.25
47-I	0.64	2.1	16
-2	0.53	2.2	13.25
62-I	0.55	2.3	13.75
-2	0.63	0.9	15.75
67-I	0.9	2.3	22.5
-2	0.91	0.8	22.75
92-I	0.36	8.9	9
-2	0.37	3.4	9.25
95-I	1.12	0.9	28
-2	1.16	3.1	29
112-I	0.49	3.9	12.25
-2	0.33	8.5	8.25

Cu	Concentration	RSD	Dilution	1 in 2	Concentration
	ppm	%	PPM	%	mg / kg
K1-1	1.11	0.9			27.75
-2	1.18	0.1			29.5
K2-1	0.47	0.4			11.75
-2	0.76	0.4			19
K3-1	0.53	0.8			13.25
-2	0.66	0.5			16.5
K4-1	0.59	0.6			14.75
-2	0.55	0.7			13.75
K5-1	0.57	0.2			14.25
-2	0.37	0.4			9.25
K6-1	0.4	0.5			10
-2	0.35	0.7			8.75
K7-1	0.26	0.4			6.5
-2	0.27	0.1			3.75
K8-1	0.17	0.7			8.5
-2	0.18	0.6			9
K9-1	0.9	0.5			45
-2	0.9	0.1			45
K10-1	1.14	0.8			57
-2	1.14	0.6			57
K11-1	0.6	0.3			30
-2	~~1.33~~	~~0.3~~	0.35	0.6	35
K12-1	0.48	0.1			24
-2	0.32	1			16
K13-1	0.55	0.3			13.75
-2	0.53	0.5			13.25
K14-1	0.51	0.7			12.75
-2	0.38	0.6			9.5
K15 -1	0.73	0.3			18.25
-2	0.61	0.2			15.25
K16-1	0.57	0.3			14.25
-2	0.32	0.4			8
K17-1	0.2	1.1			5
-2	0.22	0.2			5.5
K18-1	0.32	0.6			8
-2	0.37	0.4			9.25
K19-1	0.46	0.6			11.5
-2	0.3	0.1			7.5
K20-1	0.24	0.9			12
-2	0.19	0.1			9.5
K21-1	0.15	1.1			7.5
-2	0.16	0.4			8
K22-1	0.4	0.5			20
-2	0.94	0.2			47
K23-1	0.19	0.2			9
-2	0.22	0.5			11
K24-1	0.27	0.4			13.5

-2	0.31	0.4			15.5
K25 -1	0.27	0.3			13.5
-2	0.29	0.7			14.4
K 26 -1	0.73	0.8			36.5
-2	0.74	0.3			37
K36 -1	0.31	0.9			15.5
-2	0.31	0.5			15.5
K37 -1	1.34	0.8	0.73	3.8	73
-2	1.18	0.2			59
K38 -1	0.74	0.4			37
-2	0.89	0.7			44.5
K39 -1	1.92	0.6	1.06	0.5	106
-2	1.65	0.4	0.82	0.3	82
T2 -1	0.96	0.3			24
-2	0.93	0.5			23.25
T3 -1	1.18	0.5			29.5
-2	1.07	0.4			26.75
T4 -1	0.81	0.3			20.25
-2	0.8	0.6			20
T5 -1	0.92	0.2			23
-2	0.95	1.3			23.75
T6 -1	0.84	0.7			21
-2	0.97	0.4			24.25
T7 -1	0.9	1			22.5
-2	0.93	0.2			23.25
T8 -1	1	0.8			25
-2	1.01	0.5			25.25
T9 -1	0.69	0.4			17.25
-2	0.67	1			16.75
T10 -1	0.51	0.5			12.75
-2	0.42	0.1			10.5
A-1	0.29	0.2			7.25
-2	0.31	0.5			7.75
B-1	0.34	0.3			8.5
-2	0.33	0.5			8.25
C-1	0.28	0.9			7
-2	0.3	1.4			7.5
D-1	0.32	1			8
-2	0.3	0.2			7.5
E-1	0.86	0.4			21.5
-2	0.67	0.5			16.75
F-1	0.67	0.3			16.75
-2	0.44	0.8			11
G-1	0.29	0.7			7.25
-2	0.34	1.4			8.5
H-1	0.26	0.2			6.5
-2	0.31	0.4			7.75
I-1	0.47	0.5			11.75
-2	0.54	0.3			13.5
J-1	1.11	0.1			27.75
-2	1.17	0.3			29.25
K-1	0.34	0.5			8.5

-2	0.35	0.6		8.75
4-I	0.57	0.4		14.25
-2	0.38	0.6		9.5
9-I	0.26	0.3		6.5
-2	0.33	0.7		8.25
26-I	0.41	0.2		10.25
-2	0.34	0.2		8.5
30-I	0.68	0.2		17
-2	0.66	0.1		16.5
38-I	0.31	0.5		7.75
-2	0.42	0.3		10.5
47-I	0.35	0.5		8.75
-2	0.38	0.4		9.5
62-I	0.85	0.3		21.25
-2	0.96	0.3		24
67-I	0.43	0.6		10.75
-2	0.47	0.2		11.75
92-I	0.35	1		8.75
-2	0.37	1.2		9.25
95-I	1.13	0.5		28.25
-2	1.13	0.2		28.25
112-I	0.47	0.4		11.75
-2	0.31	1		7.75